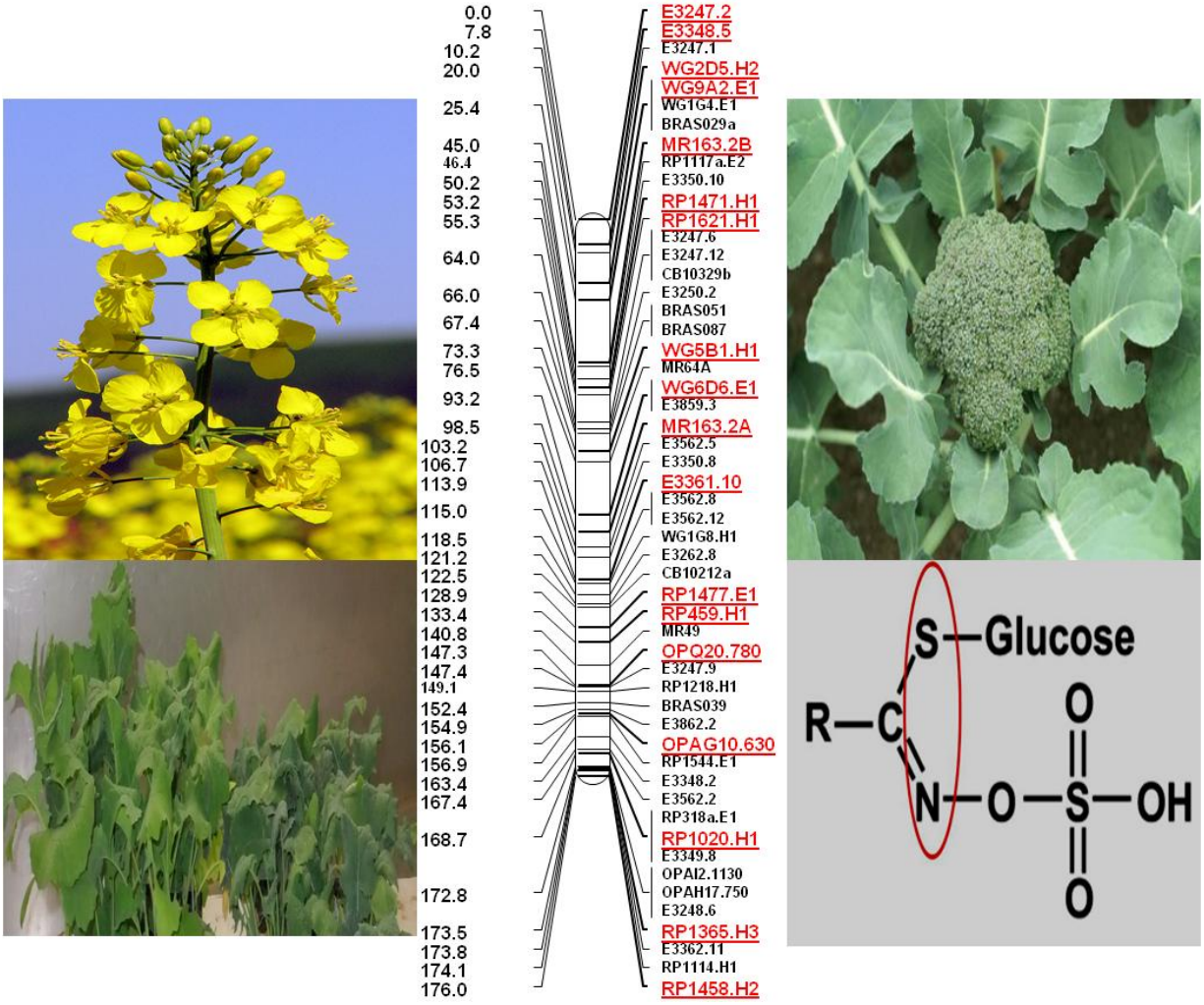
Genetic mapping of QTL controlling salt tolerance and glucosinolates in *Brassica napus* and *Brassica oleracea*



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Genetic mapping of QTL controlling salt tolerance and glucosinolates in *Brassica napus* and *Brassica oleracea*

Doctoral Dissertation

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Dedication

To my family for their moral support

To my wife and my children for their patience

Table of contents

List of tables

Chapter II: Experiments to develop a screening method and to select a suitable DH population

Chapter III: Mapping QTL for salt tolerance at seed germination in *Brassica napus* and *Brassica oleracea* doubled-haploid populations

List of figures

Chapter I: Literature Review

Chapter II: Experiments to develop a screening method and to select a suitable DH population

Chapter III: Mapping QTL for salt tolerance at seed germination in *Brassica napus* and *Brassica oleracea* doubled-haploid populations

Chapter V: Mapping QTL for salt tolerance at the young plant stage and leaf glucosinolates in a *Brassica oleracea* DH population

Chapter VI

General introduction

The *Brassicaceae* family includes many important forage crops such as *B. rapa, B. napus*, and *B. oleracea*. This family is the source of a number of oilseed (oilseed rape) and fodder crops, in addition to ornamentals such as *Cheiranthus* (wallflower), *Lobularia maritima* (sweet alysson), and *Hesperis* (rocket). *Brassica* is one of the most important genera of the *Brasicaceae,* comprising up to 100 species, including cauliflower, broccoli, brussels sprouts, turnips and various mustards (Gómez-Campo and Prakash 1999). *Brassica napus* (rapeseed, oilseed rape, Canola) is an oilseed crop of global economic importance. *Brassica napus* L. is the main European oilseed crop. The oil is used for human consumption, and in the chemical and pharmaceutical industries or as fuel (Wittkop et al. 2009). *Brassica napus* is an important crop for the human food supply and for animal feed. It originated from interspecific hybridizations between turnip rape (*Brassica rapa* L.; AA, 2n = 20) and cabbage (*Brassica oleracea* L.; CC, 2n = 18) that occurred spontaneously (Iñiguez Luy and Federico 2011) and is considered a new crop, its production beginning about 500 years ago (Gómez-Campo and Prakash 1999).

Salinity is a soil condition in which the soil includes excess soluble ions (Munns 2005). Salinity can decrease crop productivity by 20%. This reduction is attributed to the reduction in different stages from seed germination until the fruiting stage (reviewed by Ashraf et al. 2008). The deleterious effect of salinity on seed germination and plant growth is a biphasic process. The first phase is the osmotic stress which exerts a negative effect on plant growth immediately with onset of the salt stress. The second phase is ion toxicity which results from the accumulation of excess ions in the plant tissues, especially Na^+ and Cl^- (Munns and Tester 2008). Salinity tolerance varies across the plant ontogeny. Therefore, salt tolerance at one stage like seed germination is not necessarily correlated with another stage such as vegetative growth (Mano and Takeda 1997, Foolad 1999). The investigation of salt tolerance during different growth stages is necessary to disentangle the problem of salinity. *Brassica* species were ranked as moderately salt tolerant, with a superiority of the polyploid species like *B. napus* over their diploid ancestors *B. rapa* and *B. oleracea* (Ashraf et al. 2001, Ashraf 2001).

Many approaches have been proposed to relieve the detrimental effects of salinity. One of these is the technical approach, which modifies the soil to meet plant demands through the construction of efficient irrigation systems, but this is a costly and temporal solution. Another solution is to select for salt-tolerant varieties, an approach known as the biological approach (Ashraf et al. 2001). A number of methods have been employed to implement the biological approach, such as conventional breeding, which is time-consuming and laborious. Another disadvantage is the transfer of undesirable genes (reviewed by Ashraf and Foolad 2013). Thus there was a need for fast, efficient and cheap techniques such as marker assisted selection (MAS) or gene transfer. A prerequisite for MAS or gene transfer is the identification of the genomic regions harboring the causal genes, which can be achieved by the mapping of quantitative trait loci (QTL).

Glucosinolates (GSL) are plant Sulfur and Nitrogen containing secondary metabolites. More than 120 derivatives were recorded in glucosinolate-containing plant families as a result of secondary modifications (Halkier and Gershenzon 2006). Glucosinolates exhibit a wide spectrum of biological activities with both negative and positive nutritional attributes (Mithen 2001). Recently, the importance of the *Brassica* crops increased due to the discovery of their cancer-preventing compounds. Isothiocyanate sulforaphane, a derivative of 4-methylsulfinylbutyl (glucoraphanine), was found in broccoli. Sulforaphane and other isothiocyanates may stop tumor growth by cutting off the cell cycle and enhancing programmed cell death (Thornalley 2002).

So far no QTL analysis of the salt tolerance of *Brassica* species has been published (Nayidu et al. 2013). Our knowledge about the genetic control of leaf GSL is rather limited compared to that about seed GSL. Furthermore, few reports have been released about the effect of salinity on GSL variation and content. Xin et al. (2008) found that the total indolic and the total aromatic GSL increased in *Arabidopsis* under application of 150 mM NaCl. The GSL content and single components varied between different organs under salt stress with developmental stages in *Thellungiella salsuginea* under different concentrations of NaCl (Pang et al. 2012). In another study in broccoli, the GSL level was high in florets compared with newly occurring leaves, possibly due to GSL transfer through phloem tissue to another organ or *in situ* biosynthesis in florets (López-Berenguer et al. 2009).

Therefore, the present study addresses the following topics in six chapters. **The first chapter** comprises a general introduction and a literature review on the salt tolerance of the *Brassica* species and GSL. **The second chapter** shows the pre-experiments that we conducted to develop a suitable method to test *Brassica* mapping populations at the juvenile stage in the greenhouse, in order to determine the most suitable salt concentration and to select a suitable mapping population based on the performance of the parents. **The third chapter** shows the analysis of the genetic variation in seed germination under control and salt stress conditions in doubled-haploid (DH) mapping populations of *B. napus* and *B. oleracea* and identifies the QTL that govern the genetic variation in seed germination under control and salt stress conditions. **The fourth chapter** addresses the effect of salinity on *B. napus* in a DH mapping population under 200 mM NaCl at the young plant stage, and identifies the QTL for traits measured under both growth conditions. Additionally, we analyze the leaf GSL variation under control and salt stress and map the QTL that control the variation in leaf GSL profile and content under both growth conditions. **The fifth chapter** seeks to determine the effect of salinity on *B. oleracea* in the DH mapping population Bo1TBDH under treatment with 100 mM NaCl at the young plant stage and to identify the QTL for traits measured under both growth conditions, i.e. control and salinity. Furthermore, this chapter investigates the variation in leaf GSL under control conditions and salt stress and maps the QTL for this variation. **The sixth chapter** includes a general discussion of the results.

The main objectives of the present study are

1. To establish a suitable method to test a large number of genotypes for salinity in the greenhouse and to select a mapping population based on the performance of its parents.
2. To address the effect of salinity on seed germination in DH mapping populations of *B. napus* and *B. oleracea* and to identify the QTL that control the variation in the measured germination parameters.
3. To analyse the effect of salinity on plant growth at the juvenile stage and to identify the QTL that control the variation in different traits under control and salt stress.
4. To measure the variation in leaf GSL under control and salt stress and to identify the QTL that underlie the genetic variation in leaf GSL under control and salt stress.

Chapter I

Literature review

1.1 The family *Brassicaceae*

The *Brassicaceae* exhibit a cosmopolitan distribution, although certain regions of the world have a greater density of genera. Some members of this family are found in most parts of the world, but they are mainly concentrated in the northern temperate region, especially in the countries surrounding the Mediterranean basin and in southwestern and central Asia (Gómez-Campo and Prakash 1999). The *Brassicaceae* are widely distributed all over the world and comprise approximately 338 genera and 3709 species (Warwick et al. 2011). The most important diversification centers are found in The Irano-Turanian region with ca. 150 genera and ca. 900 species with 530 endemics and in the Mediterranean region with ca. 113 genera and ca. 630 species with 290 endemics. The Saharo-Sindian region includes 65 genera and 180 species with 62 endemics and North America has ca. 99 genera and 778 species with 600 endemics. This smaller species diversity is continued in the southern hemisphere: South America with 40 genera and 340 species; Southern Africa with 15 genera and at least 100 species; and Australia and New Zealand with 19 genera and 114 species (Lysak and Koch 2011). The *Brassicaceae* include many important forage crops, such as *B. rapa, B. napus*, and *B. oleracea*. This family is the source of a number of oilseed (oilseed rape) and fodder crops, in addition to ornamentals such as *Cheiranthus* (wallflower), *Lobularia maritima* (sweet alysson), and *Hesperis* (rocket). The genus *Brassica* is one of the most important genera of the *Brasicaceae,* comprising up to 100 species, including cauliflower, broccoli, brussels, sprouts, turnips and various mustards (Gómez-Campo and Prakash 1999).

Genomic relationships between the diploid and polyploid Brassica species

The relationships between the different cultivated *Brassica* genomes that characterize the different species of the *Brassica* genus have been addressed in a cytological work (Morinaga 1934). The species *B. napus* (2n = 38, AACC), *B. juncea* (2n = 36, AABB), and *B. carinata* (2n = 34, BBCC) are amphidiploids, comprising pairs of chromosome sets from their diploid ancestors *B. rapa* (2n = 20, AA), *B. oleracea* (2n = 18, CC) and *B. nigra* (2n = 16, BB), (Figure 1). This hypothesis was verified (U 1935) in a successful resynthesis of *B. napus* by crossing *B. rapa* with *B. oleracea*. The resynthesis of *B. juncea* and *B. carinata* was done later by (Frandsen 1943, 1947).

Brassica napus

Brassica napus (rapeseed, oilseed rape, Canola) is an oilseed crop of global economic importance. *Brassica napus* is the main European oilseed crop. The oil is used for human consumption, and in the chemical and pharmaceutical industries or as fuel (Wittkop et al. 2009). *Brassica napus* was originated from a spontaneous interspecific hybridization between turnip rape (*Brassica rapa* L.; AA, 2n = 20) and cabbage (*Brassica oleracea* L.; CC, 2n = 18), (Iñiguez-Luy and Federico 2011).

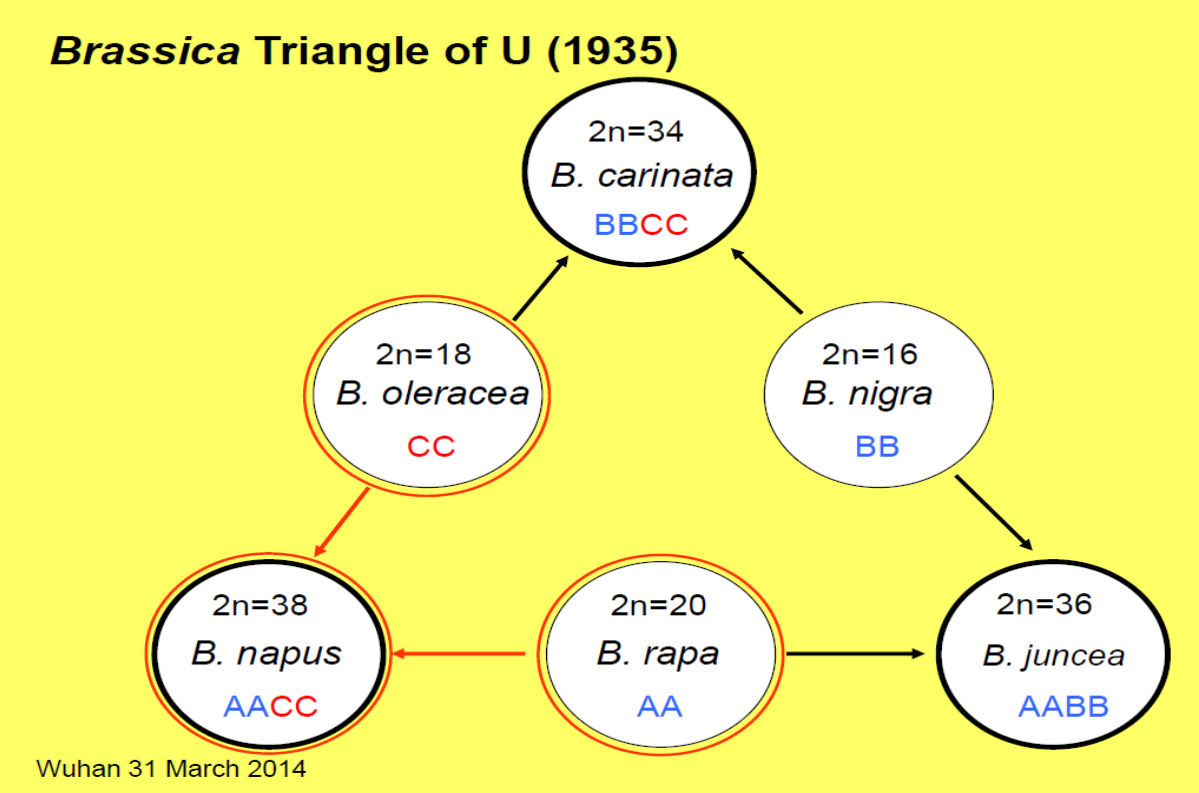


Figure I-1: Triangle of U shows the evolution of the amphidiploids *Brassica* species from their diploid progenitors according (Morinaga 1934 and U 1935)

Brassica napus is thought to be a relatively new species that was developed only 500 years ago (Gómez-Campo and Prakash 1999). Based on chloroplast DNA investigation, Allender and King (2010) reported that there were two species, *B. rapa* (brocoletto crop type) from southern Italy, and a second one grown in Portugal. One of them would have been hybridized with *B. oleracea*. It is apparent that the former was grown close to *B. oleracea* crops like kale, cabbage and broccoli, which smoothed the hybridization with *B. oleracea* and led to the evolution of *B. napus*. A clear differentiation between *B. rapa*, *B. oleracea* and *B. napus* using highly diverse chloroplast microsatellite markers was unsuccessful because *B. napus* formed its own cluster (Zamani-Nour et al. 2013). Becker et al. (1995) reported that the modern elite varieties possess low levels of diversity due to few hybridization events together with the occurrence of two bottlenecks during *B. napus* breeding. *Brassica napus* has achieved economic importance as an oilseed crop in the past 40 years following an intensive breeding program. This breeding program aimed at increasing yields and decreasing the high levels of erucic acid in the seed oil to make it relevant for human consumption. Moreover, reducing the high levels of aliphatic glucosinolate in the residue after oil extraction made it more relevant and safe for animal feed (Wittkop et al. 2009).

Brassica oleracea

Representing the cole crops, this species is a member of the CC genome cohort, which includes several interfertile species, i.e. *Brassica cretica*, *Brassica hilarionis*, *Brassica incana*, *Brassica insularis*, *Brassica macrocarpa*, *Brassica montana*,

Brassica rupestris, and *Brassica villosa* (Warwick 2011). The wild forms of *B. oleracea* have been found in the coastal areas of western Europe and *Brassica bourgeaui* in the Canary Islands (Warwick 2011). The cultivated forms of *B. oleracea* can be subdivided into different classes based on their edible parts. In kales (var. *viridis*, var. *costata*, var. *medullosa*, var. *sabellica*) and branching bush kales (var. *ramosa*), the edible part is the foliage leaves. The tightly packed leaves are the edible part of cabbages (var. *capitata* and var. *sabauda*) *and* brussels sprouts (var. *gemmifera*). The above-ground, thickened stem is the edible part of kohlrabi (var. *gongyloides*). Cauliflower and broccoli (var. *botrytis* and var. *italica*) are cultivated for their thickened edible inflorescences (Warwick 2011). These diverse morphotypes provide an ideal material to investigate human-directed evolution (artificial selection) and the processes involved in domestication. *Brassica oleracea* genome structure is not only related to the vegetable crops that it represents itself, but also one of the parents of *B. napus*, oilseed rape, the second edible oil source among the oilseed crops (Warwick 2011). Song et al. (1990) reported that the cultivated forms have a monophyletic origin from the same progenitor. Probably it was a leafy kale from which the other cultivated types diverged. Moreover, this study revealed that other wild CC genomes cytodeme, such as *B. insularis* and *B. incana,* may also share the diversity of cultivated *B. oleracea*. *Brassica* vegetables appear to protect against cancer and heart disease, principally due to the presence of glucosinolates which are secondary metabolites that break down into isothiocyanate (sulphoraphane). These Sulphur-containing compounds act as anti-cancer agents, inducing detoxification enzymes and limiting tumors growth by nullifying carcinogenic- chemicals in cells (Talalay et al. 1995).

1.2 Salinity

Soil is classified as saline when the electric conductivity (ECe) exceeds 4 EC (40 mM NaCl), (USDA Salinity Laboratory 2005). Worldwide, more than 800 million hectares, which represent 6% of the earth, are salt-affected lands (USDA Salinity Laboratory 2005). The major factors reducing crop productivity are abiotic stresses (Boyer 1982). Yield losses due to individual abiotic stresses were estimated as 17% for drought, 20% for salinity, 40% for high temperature stress and 15% for frost (Ashraf et al. 2008). With regard to salinity, plants can be categorized into two categories, tolerant plants (halophytes) and non-tolerant plants (glycophytes). Halophytes can withstand high amounts of Na^+ and Cl^- due to anatomical adaptations and intracellular partitioning. Dicotyledonous halophytes developed two types of anatomical adaptations: an increase in cell size due to increased vacuole volume (succulence), or the exclusion of Na^+ and Cl^- by salt glands or bladders (Flowers et al. 1977).

Types of salinity

Natural or primary salinity is the accumulation of salts over the long term, via two processes. The first is soil erosion, which releases many soluble salts. The second is precipitation of salts carried by rains and wind. Secondary or human-induced salinity results from human activities such as land clearing, replacement of natural vegetation with crops, and/or use of poor irrigation water (Munns 2005).

Phases of salt stress and mechanisms of response

Plants have developed numerous defense strategies to overcome salt stress. These strategies include minimizing exposure, avoidance, and/or tolerance.

Minimizing exposure is achieved by inducing early flowering and a short life cycle. Avoidance is accomplished through exclusion of salt ions, increasing water uptake and decreasing water loss by closing stomata to prevent or decrease water evaporation. Tolerance becomes the alternative when the other defenses against salt stress are impossible (Chaves et al. 2003). Plant salt tolerance involves osmotic adjustment, exclusion of excess ions and sequestering the excess ions in the vacuole. When these mechanisms are inadequate, plants develop peripheral defense mechanisms such as detoxification of reactive oxygen species (Munns and Tester 2008). The abilities of plants to withstand salt stress depend on the species, period of exposure, the concentration of the salt and the growth conditions (Ashraf and Foolad 2013). The physiological adaptations of plants to salt stress are summarized in Figure 2 (Nayidu et al. 2013).

Effect of salinity on seed germination and plant growth

Seed germination and seedling establishment are two crucial steps in the life cycle of plants. The absence of optimum plant germination causes a reduction in plant density, which may result in yield reduction (El-Hendawy et al. 2011). Salt stress delays germination and increases its time-course (Foolad and Jones 1991). High concentrations of salts surrounding roots impair seed germination and crop establishment (Fowlers 1991).

High salinity reduces plant growth and development dramatically. This reduction could be due to osmotic stress or adverse specific ion toxicity (Munns and Tester 2008). The steep build-up of sodium and chloride perturbs membrane integrity and function, and causes nutrient ion imbalances. High levels of sodium or chloride impair the uptake of essential minerals such as potassium, nitrate or phosphate (Grattan and Grieve 1999). Osmotic stress has a more severe impact than ion-specific stress. The former begins much earlier, especially under low and moderate salt concentrations. It is accompanied by a slower development of new leaves, lateral buds and fewer lateral branches. Ion-specific stress on the other hand more strongly affects sensitive plants that are not able to control salt uptake. The death of older leaves is a sign of the second phase (Munns and Tester 2008).

Effect of salinity on photosynthesis

Stomata closure is the immediate and fastest response of the entire plant to osmotic stress. The reduction of photosynthesis is accompanied by other changes in the leaf anatomy and carbohydrate metabolism. Under salt stress the leaves become smaller and thicker, resulting in high chloroplast number per leaf area (Fricke et al. 2004). The accumulation of unused carbohydrates generates feedback signals to slow down the photosynthesis (Paul and Foyer 2001). The reduction in photosynthesis might be attributed to the inhibition of cytosolic enzymes, which catalyze the carbohydrate metabolism, or to the accumulation of Na^+ and Cl^- ions in chloroplasts (Munns and Tester 2008).

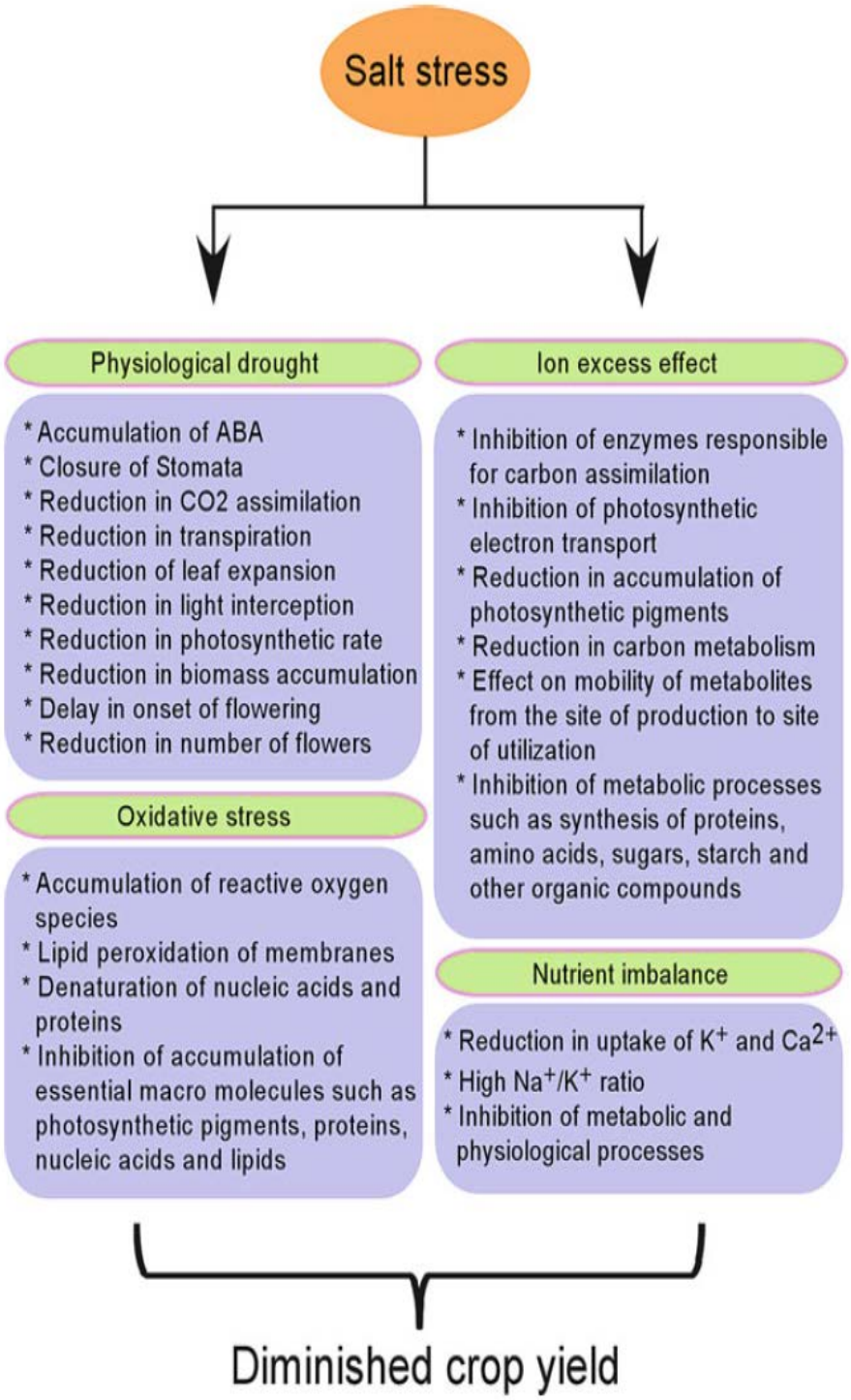


Figure I-2: Summary of salinity detrimental effects on plant growth

Source: Nayidu et al. 2013

Effect of salinity on seed oil quantity and quality

In oilseed crops, the quality of seed oil depends on the composition of fatty acids such as palmitic, stearic, oleic and linoleic acid. Salinity directly inhibits enzymes, such as glyoxysomal catalase, malate synthase, isocitratelyase and oleatedesaturase. These enzymes regulate fatty acid biosynthesis and modification. For instance, sunflower plants grown on saline irrigation water exhibited a progressive increase in oleic acid content and a decrease in linoleic acid level (Nayidu et al. 2013).

Strategies to solve the salinity problem

There are two major scenarios to minimize the detrimental effects of high soil or water salinity. Both of these strategies could be applied to maintain sustainable crop production in the presence of high salinity (Epstein et al. 1980). The first is a technical approach and involves large engineering schemes for reclamation, drainage and irrigation with high-quality water. Although these practices have had success in some areas, their costs were high. Moreover, their solution to the problem is often only temporary. The second approach is a biological one employing biological solutions based on the use or development of salt-tolerant plants (Ashraf et al. 2008). Another biological solution is the domestication of halophytes (Flowers 2004). The biological approach encompasses several steps; development of efficient screening tools for selection and evaluation of specific traits. These steps include estimating the salt tolerance of plants at different developmental stages, investigating the biological mechanisms that control salt tolerance, and developing efficient direct or indirect breeding methods to transfer the tolerance genes (Ashraf and Foolad 2013). Nobel and Rogers (1992) observed that the somewhat limited success in producing salt-tolerant genotypes is due to many factors, including the polygenic nature of salinity, the lack of efficient evaluating and selecting criteria, and a limited understanding of the mechanisms that regulate salt tolerance.

1.3 Salt tolerance in *Brassica*

Salinity reduces the growth, yield, and oil production of *Brassica* species. In *Brassica* species, seed germination and early seedling are the most sensitive stages (Ashraf and Harris 2004). There is a potential inter- and intraspecific variation in salt tolerance among the *Brassica* species. This variation can be exploited through selection and breeding to ameliorate salt tolerance. *Brassica* was classified as moderately salt tolerant with a superiority of amphidiploids species over the diploid species (Mailk 1990; He and Cramer 1992). Ashraf and Harris (2004) reported that the amphidiploids species are more salt tolerant compared to the diploid species, and that the salt tolerance of amphidiploids species was inherited from the diploid progenitors. This is in agreement with the findings of Stebbins (1966), who reported that the polyploid species can withstand adverse environmental stresses better than their respective diploid ancestors.

Improving salt tolerance in *Brassica*

Several approaches were harnessed to enhance salinity tolerance in *Brassica.* Conventional breeding approaches have allowed the production of many salt-tolerant varieties of *Brassica*. In India, salt-tolerant varieties of *Brassica juncea* were developed (Purty et al. 2008). Salt tolerance of the *Brassica* tribe was analysed by

estimating the whole plant growth, proline accumulation, K^+/Na^+ ratio and electrolyte leakage (Purty et al. 2008). *Brassica juncea* var. CS52 was recommended for cultivation in saline soils. More lines and varieties were developed in other species, like *B. carinata* and *B. rapa.* The names of lines and varieties, selection parameters and the authors are summarized (Appendix 5).

Jain et al. (1990) developed salt-tolerant lines of *B. Juncea* using an *in vitro* approach. Out of 2,650 cotyledons cultured in high salt level media, three calli survived and resurrected shoots. When these three lines were tested on salt-enriched medium, two of them flowered and produced seeds. The third displayed abnormal morphological features and was sterile. Kirti et al. (1991a) produced sodium chloride-tolerant lines from somatic embryos of *B. juncea* line RLM198. In this experiment, the plants, which were regenerated from the tolerant lines, were tested for salt tolerance by measuring esterase isozyme pattern and proline accumulation. The tolerant calli accumulated higher amounts of proline than the sensitive ones. Elavumoottil et al. (2003) developed salt-tolerant calli and cell suspension from *B. oleracea* var. *botrytis.* The salt-tolerant ones showed high sucrose content and reduced sugars and sucrose synthase.

Fast and efficient techniques were used to speed up the development of salt-tolerant lines by gene transfer to modify metabolic pathways or to engineer ions exchange. For example, the expression of the bacterial codA gene in *B. napus* improved the salt tolerance. The transgenic lines showed a significant increase in seed germination and seedling under salinity compared to the wild ones (Huang et al. 2000; Prasad et al. 2000). Zhang et al. (2001) transformed *B. napus* by the AtNHX gene, coding for a vacuolar Na^+/H^+ antiporter from *A. thaliana*. Whereas the growth of the wild lines was markedly reduced, the transformed lines were able to continue growing, flowering and producing seeds. Another successful example was the production of high NaCl-tolerant cultivars of *Brassica oleracea* var. *capitata* cultivar 'Golden Acre', by transformation with a bacterial *bet*A gene (Bhattacharya et al. 2004). The overexpression of the (PR)-10 family derived from Pea in *B. napus* improved seed germination in the presence of NaCl (Srivastava et. al. 2004). Park et al. (2005) stated that the expression of *B. napus* late embryogenesis (LEA) group 3 abundant protein genes boosted salinity and drought tolerance of the Chinese cabbage *B. rapa*. *Brassica juncea* was able to withstand high salinity levels of up to 300 mM NaCl after being transformed with the PgNHX1 gene from *Pennisetum glaucum* (Rajagopal et al. 2007). The overexpression of *Arabidopsis* Dehydration-responsive element binding factor 2C (DREB2C) in *B. napus* ameliorated the salt tolerance. The overexpression of this gene increased chlorophyll content and reduced water loss in the transformed types compared to the wild types (Song et al. 2014). These reports demonstrate the considerable increase in salt tolerance achieved by single gene overexpression, despite the fact that salt tolerance is a polygenic trait.

1.4 QTL and Salinity

A current approach to increase the efficiency of selection and breeding for complex traits such as salt tolerance is indirect selection using genetic markers. This target requires identifying these genetic markers, which are linked to the traits of interest. The use of quantitative trait loci (QTL) is a powerful method for finding the link

between the genetic markers mapped on the chromosomes and the traits of interest (Ashraf and Foolad 2013).

The QTL controlling salt tolerance during different growth stages

Several QTL controlling seed germination under salinity conditions were mapped. Fourteen QTL controlling salt tolerance at germination and during vegetative growth have been detected in tomato (Foolad and Chen 1999). In wheat, several QTL were mapped these QTL increase biomass, root length, shoot length, proline and chlorophyll content during germination and seedling survival (Ma et al. 2007). Several QTL were identified for salt tolerance at the reproductive stage of tomato (Villalta et al. 2007). These studies suggest that an understanding of the complexity of salt tolerance can be achieved through investigation of salt tolerance at different developmental stages.

QTL for ion uptake

Several QTL regulating ion uptake were identified in different crops. For example, four QTL for the ion transport were detected in wheat (Huang et al. 2006). In wheat, the QTL Kna1 was identified for Na^+/K^+ selectivity (Dubcovsky et al. 1996). In rice, several QTL were QTL for Na^+/K^+ homeostasis in roots and shoots (Ming-zhe et al. 2005, Sabouri and Sabouri 2008). Fourteen QTL for mineral ions uptake, including one QTL for Na^+ exclusion, were mapped in sunflower (Lexer et al. 2003).

1.5 Glucosinolates

Glucosinolates are plant Sulfur and Nitrogen containing secondary metabolites. Glucosinolates are distributed in 16 dicotyledonous plant families. The *Brassicaceae* family, including important *Brassica* crops such as oilseed rape (*Brassica napus*), cabbage (*Brassica oleracea*) and the model plant (*Arabidopsis thaliana*), is well-known for the presence of glucosinolates (Mithen 2001). More than 120 derivatives were recorded in glucosinolate-containing plant families as a result of secondary modifications (Mithen 2001; Halkier and Gershenzon 2006). The structure of the glucosinolate molecule is made up mainly of a β-thioglucose moiety, a sulphonated oxime core and alterable side chains. This moiety is derived from different amino acids: alanine, valine, leucine, isoleucine, phenylalanine, tyrosine and tryptophan. The biosynthesis of glucosinolates is a triphasic process. The first step is the elongation of the amino acid side chain by adding a methylene group. The second and the third steps are the formation of the core structure, and the modification of the side chain (Halkier and Gershenzon 2006, Sønderby et al. 2010). Glucosinolates are classified into three classes according to the precursor amino acid. The aliphatic glucosinolates are derived mainly from methionine. The indolic glucosinolates are derived from tryptophan. The aromatic glucosinolates are derived from phenylalanine (Halkier and Gershenzon 2006).

Importance of glucosinolates

Glucosinolates (GSL) exhibit a wide spectrum of biological activities, such as its effective role in plant-herbivore interactions. Glucosinolates degradation products repel herbivores such as birds, mammals and molluscs (Mithen 2001). The repellant ability of glucosinolates depends basically on their composition and population diversity of the herbivores (Giamoustaris and Mithen 1995). The chemical structure of the glucosinolate products plays a striking role in their biological activity (Mithen 2001). Glucosinolate are vacuole-sequestered metabolites and chemically stable (Koroleva et al. 2000). After cell injury, pathogen

infection, or pest attack, GSL come in contact with the cytosolic hydrolases (myrosinases). This enzyme hydrolyses GSL and highly toxic products are released. These products generate the plant-herbivore interactions (Bennett and Wallsgrove 1994).

Recently, the importance of *Brassica* crops increased due to recognition of their cancer-preventing components, released by GSL degradation. These GSL hydrolysis products induce phase II detoxification enzymes, or hinder tumor growth by enhancing programmed cell death (Thornalley 2002). The isothiocyanate sulforaphane, a derivative of glucoraphanine (RAA), shows a potential influence against gastritis and stomach cancer (Fahey et al 2002). In animal nutrition, the presence of high levels of GSL in the seeds of cruciferous oilseed crops markedly reduces the quality of the seed meal left after oil extraction. This is mainly due to the presence of certain GSL compounds such as progoitrin. Thus feeding rapeseed meal with high levels of progoitrin causes thyroid gland disorder, kidney and liver failure (Mithen 2001).

It has been observed that myrosinase activity is high, particularly in developing tissue, which suggests that GSL influences plant growth (Husebye et al 2000). The involvement of glucosinolates-myrosinase complex in plant growth was reported in *Arabidopsis.* The mutant lines lacking the gene that regulates the short chain glucosinolates biosynthesis (CYP79F1) showed a strong dwarf, bushy and semi-sterile phenotype (Reintanz et al. 2001).

Glucosinolates in *Brassica* species

In the *Brassica*ceae family, about 30-40 different glucosinolates are produced by each species, the methionine-derived (aliphatic GSL) being predominant (Halkier and Gershenzon 2006). *Brassica napus* has a restricted and unique aliphatic glucosinolate profile. It contains butenyl and pentenyl glucosinolates and their hydroxylated counterparts (Mithen 2001). Conversely, the GSL composition of *B. rapa* and *B. oleracea*, the putative donors of A and C genomes of *B. napus*, have different profiles. *Brassica oleracea* may contain propenyl and/or butenyl glucosinolates, while *B. rapa* contains butenyl and often pentenyl glucosinolates. Both species may also have significant quantities of methylthioalkyl and methylsulphinylalkyl homologues. This seems to be plentiful, especially within the cultivated forms of *B. oleracea* (Magrath et al 1993). Synthetic *B. napus* lines, which were derived from *B. rapa* and *B. oleracea* by interspecific hybridization or by embryo rescue techniques have different GSL profiles to those of the natural forms of *B. napus.* This is due to the interaction between the A genome and the C genome (Magrath et al. 1993). It was reported that in *Brassica* species, GSL represent 1.7% to 8.0% of total plant sulfur content (reviewed by Falk et al. 2007).

Great attention has been given to reducing the glucosinolate content of the seeds of oilseed rape *B. napus*. This aim has been successfully accomplished via the introgression of alleles from the low seed GSL cultivar Bronowski into Canadian spring rape cultivars and then into European winter rape cultivars. This integration led to the development of the current 00 cultivars. Cultivars or lines with low seed GSL were selected successfully without pernicious effects on the GSL content of other tissues (Mithen 2001). The genotypes with low seed GSL do not necessarily have low glucosinolate content in vegetative tissues. Lines with high glucosinolate

content may have low/high leaf glucosinolates content. There was no correlation between the glucosinolate content of leaves, stems, and seeds (Li et al 1999; Cleemput and Becker 2011). Thus, GSL synthesis and accumulation seems to be under tissue-specific control, and the effect of mutation which blocks accumulation of glucosinolates in seeds is tissue-restricted (Li et al 1999). Similarly, tissues-specific profile was observed in a set of Chinese lines that were utilized to produce synthetic *B. napus* (Giamoustaris and Mithen 1995).

Glucosinolates and quantitative trait loci

In different plant materials, mostly four QTL on *B. napus* chromosomes N9, N12, N17, and N19 were detected independently (Uzunova et al. 1995; Quijada et al. 2006). These findings indicate that these QTL are major loci for seed GSL content (Hasan et al. 2008). Howell et al. (2003) found that the QTL on N9, N12 and N19 were homoeologous loci. In *Arabidopsis*, a number of QTL controlling the accumulation of aliphatic, aromatic, and indole glucosinolates in leaves and seeds were identified in *Landsberg erecta* (Ler) X Cape Verde Islands (Cvi-0) recombinant inbred lines. In another study on the same plant material, Kliebenstein et al. (2001b) mapped six QTL that control the total aliphatic GSL accumulation. Of these, two QTL harbored the genes GS-Elong and GS-AOP. Additionally, six QTL controlling the total indolic GSL, and three QTL controlling the aromatic GSL accumulation were mapped.

The interaction between glucosinolates and abiotic stresses

The role of glucosinolates in biotic stresses such as insect attack and pathogens has been intensively studied. In contrast, the role of GSL under abiotic stressors such as light, drought, salinity and heat is still vague. Several environmental factors affect the concentration and composition of GSL, including light, water supply, temperature and salinity (Rosa et al. 1998; Qasim et al. 2003; Velasco et al. 2007; López-Berenguer et al. 2008; Mewis et al. 2012). In *B. rapa,* the activity of the transcription factors involved in GSL synthesis has been altered under different growth regimes (Justen 2010). The HY5 transcription factor, which is involved in the assimilation of sulfur-containing metabolites, down regulated the transcription of aliphatic GSL-related MYB transcription factors and enhanced indolic GSL-related MYB transcription factors (Huseby et al. 2013). These results suggest a cross-talk between GSL and the sulfur-containing assimilates of biosynthesis.

What can glucosinolates do under abiotic stresses?

Plants cannot escape external stressors due to their immobile nature. Therefore, they developed different mechanisms to face these stressors. One of these is the redistribution of resources among the secondary metabolites like GSL (Falk et al. 2007). Under sub-optimal growth conditions, GSL are multifunctional. Under nutrient deficiency growth conditions, in particular, Sulfur and Nitrogen, plants tend to catabolize GSL to use the released S and N in the primary metabolic processes (Falk et al. 2007). The highest activity of myrosinase was monitored in the guard cells to release isothiocyanates (AITCs). The AITCs impair the influx of K^+ into the guard cells, keeping them flaccid. This action induces stomatal closure, reduces the loss of water and fungi infections (Zhao et al. 2008). Khokon et al. (2011) found that in *Arabidopsis,* the AITCs induced the production of ROS and nitric oxide. These products act as secondary messengers in the ABA-dependent stomatal closure. The indolic GSL, glucobrassicin (GBC), induces the biosynthesis of auxins. These

auxins stimulate root growth under sub-optimal growth conditions, ensuring efficient uptake of nutrients (reviewed by Falk et al. 2007). These findings indicate that GSL may act as a secondary messenger to activate other signaling pathways.

Salinity effect on GSL

López-Berenguer et al. (2008) hypothesized that the accumulation of GSL in *B. oleracea* under salt stress plays a role in the osmotic adjustment. In *Thellungiella salsuginea,* the GSL concentration increased upon exposure to 200 mM NaCl, but with 300 mM there was a dramatic reduction in GSL levels (Pang et al. 2012). Likewise, Guo et al. (2013) observed a 2.1-fold increase in sulforaphane, one of the products of glucoraphanin hydrolysis under 100 mM NaCl in *Brassica oleracea* var. italica cv. Youxiu. Xin et al. (2008) found increases in the total indolic and aromatic GSL in *Arabidopsis* upon 150 mm NaCl. The GSL content and single components varied between different organs under salt stress in the developmental stages of *Thellungiella salsuginea* under different concentrations of NaCl (Pang et al. 2012).

The depletion in GSL content under stressful growth conditions might be due to many factors, such as the degradation of GSL to release the stored elements like Sulfur and Nitrogen, the redistribution of GSL within different organs to ensure certain defense levels. Another reason might also be a strategic use of sources, e.g. when plants experience stresses they tend to invest more energy in primary metabolism to ensure continuous growth. Sometimes the biosynthesis and translocation of GSL is costly. Another possibility is the hydrolysis of GSL by myrosinase after leakage of GSL from the vacuole (reviewed by Martinez-Ballesta et al. 2013).

Chapter II

Experiments to develop a screening method and to select a suitable DH population

Consecutive experiments were conducted to develop an applicable method and to select the suitable doubled-haploid population (DH) population. The selection of the DH population was based on the performance of its parental lines under salt stress. To address this point the genetic variation in salt tolerance of genetically diverse genotypes was evaluated. Each couple of these genotypes represented the parental lines of a DH mapping population. These parental lines are available at the Department of Crop Sciences, Georg-August-Universität Göttingen.

2.1 The objectives of this chapter are to

(1) develop a method in which a high number of genotypes could be tested.
(2) detect the optimal concentration and parameters to distinguish between the salt-tolerant and salt-sensitive genotypes.
(3) select two parental lines that varied significantly in salt tolerance, from which a DH population was derived.

Different methods were developed by other researchers, for example, Ashraf et al. (2001) tested the performance of six species of *Brassica*: *Brassica oleracea, B. rapa, B. nigra, B. napus, B. Juncea and B. carinata* in pot experiment. Each pot was filled with 7.52 kg well-washed sand. The six genotypes were tested. The control treatment was full-strength Hoagland solution. The salt treatments were 100 mM NaCl and 200 mM NaCl supplemented with full-strength Hoagland solution. El Hendawy et al. (2005b) tested the effect of different salt concentrations on eight varieties of Egyptian wheat. These varieties were tested in a greenhouse experiment under control conditions (watering with tap water), and watering with different concentrations of NaCl (50 mM, 100 mM and 150 mM). Loamy soil was filled into 7-litre pots; the soil water content for each treatment was adjusted to 25% using tap water for control or the respective salt concentration for saline conditions. During the experiment the pots were weighed daily and the soil-water content was adjusted by adding tap water as needed. Tunçtürk et al. (2011) examined the performance of 12 *B. napus* cultivars. In a greenhouse experiment, the cultivars were tested in 4-litre pots filled with loamy soil collected from the field. The control treatment was tap water and the salt stress was 150 mM NaCl.

In the present study, the above-described methods were not used because these methods were applied to test only a few genotypes. The application of these methods to test the DH populations which include hundreds of genotypes would be laborious, time- and space-consuming. Therefore; we decided to develop a new method to phenotype a high number of genotypes for salt tolerance in a greenhouse experiment. The parental lines were tested using the flooding method; this method will be described in detail below. The aims of the first experiment were to reduce the number of genotypes and to test the suitability of the flooding method to evaluate all of the lines of the DH population, as well as to discover the advantages and disadvantages of this method.

2.2 Experiment 1: Effect of salinity on the young plant growth of 13 Brassica napus parental lines.

In this experiment the fresh weight (FW) and dry weight (DW) were the parameters to select the parents; these revealed significant differences.

2.2.1 Materials and methods

The experiment was conducted according to Abel et al. (2006), with minor modifications. Thirteen genotypes: Alesi, H30, Mansholts, Samourai, Sollux, Gaoyou, Sansibar, Oase, Express, R53, Digger, DH14 and L16 were tested in the greenhouse. Six seeds of each genotype were sown in 7x7-cm pots filled with a soil mix of 50% composite and 50% sand. The soil and sand were sieved through a 5-mM mesh and dried for 24 h at 105°C. The pots were randomized on tables in a complete block design, where each genotype was represented by two pots in each replication. Each pot was watered by 100 ml tap water. After seedling emergence the soil was kept humid by overhead spraying for one week.

At day 12, plants were thinned into two plants per pot. The plants were watered every 2^{nd} or 3^{rd} day by placing them for 1 h into a bowl filled with tap water, ca 3-4 cm high. Plants were grown without saline solution till they had four leaves, around two weeks after sowing. The control plants continued to be watered with tap water until harvest. The salt-stressed plants were treated with two different salt concentrations. For acclimation, the saline concentrations were elevated gradually from the 1^{st} day to the 4^{th} day, to the final concentrations of 200 mM and 300 mM, respectively. The saline treatment continued for two weeks by applying saline solution via dipping into the bowl as described above. At day 28, the plants were harvested, whereby the shoot system was divided into different parts; 1^{st} + 2^{nd} leaves, 3^{rd} and 4^{th} leaves, hypocotyl and sprouts. The FW for each part was measured immediately, and the leaf area was measured. For DW estimation samples were dried for 72 h at 60 °C.

2.2.2 Results

A significant reduction in FW and DW was observed for all parts under both the 200 mM NaCl and 300 mM NaCl treatment compared to the controls. However, six parental lines, Mansholts, Samourai, Sollux, Gaoyou, Alesi and H30, showed higher biomasses compared to the other genotypes (Figures 1 and 2). Leaf chlorolysis, leaves dropping and plant loss were observed, especially under 300 mM NaCl.

2.2.3 Conclusion

The flooding method was suitable for phenotyping plants under salt stress in greenhouse experiments. The six parental lines of Mansholts, Samourai, Sollux, Gaoyou, Alesi and H30 were included in the following experiment. We also concluded that fertilization would be necessary to avoid the above-mentioned problems of leaf chlorolysis, leaf-drop and plant loss. The 200 mM NaCl treatment was more relevant than the 300 mM NaCl condition. Leaf area results were valuable, but this parameter made it impossible to harvest the whole plants at the same time. Moreover, the measurement process took too long, thus, this parameter was not considered in the next experiments.

The advantages of the flooding method

- No microenvironment differences because all genotypes were irrigated together, not independently.
- No logging or water deficiency because all pots were immersed in the tap water or in the saline solution. This method allows the plants to take up the optimum amount of water.

The disadvantages of the flooding method

- After watering the discharge of the rest of the water is laborious.
- The water and saline solution should be enriched with a fertilizer.

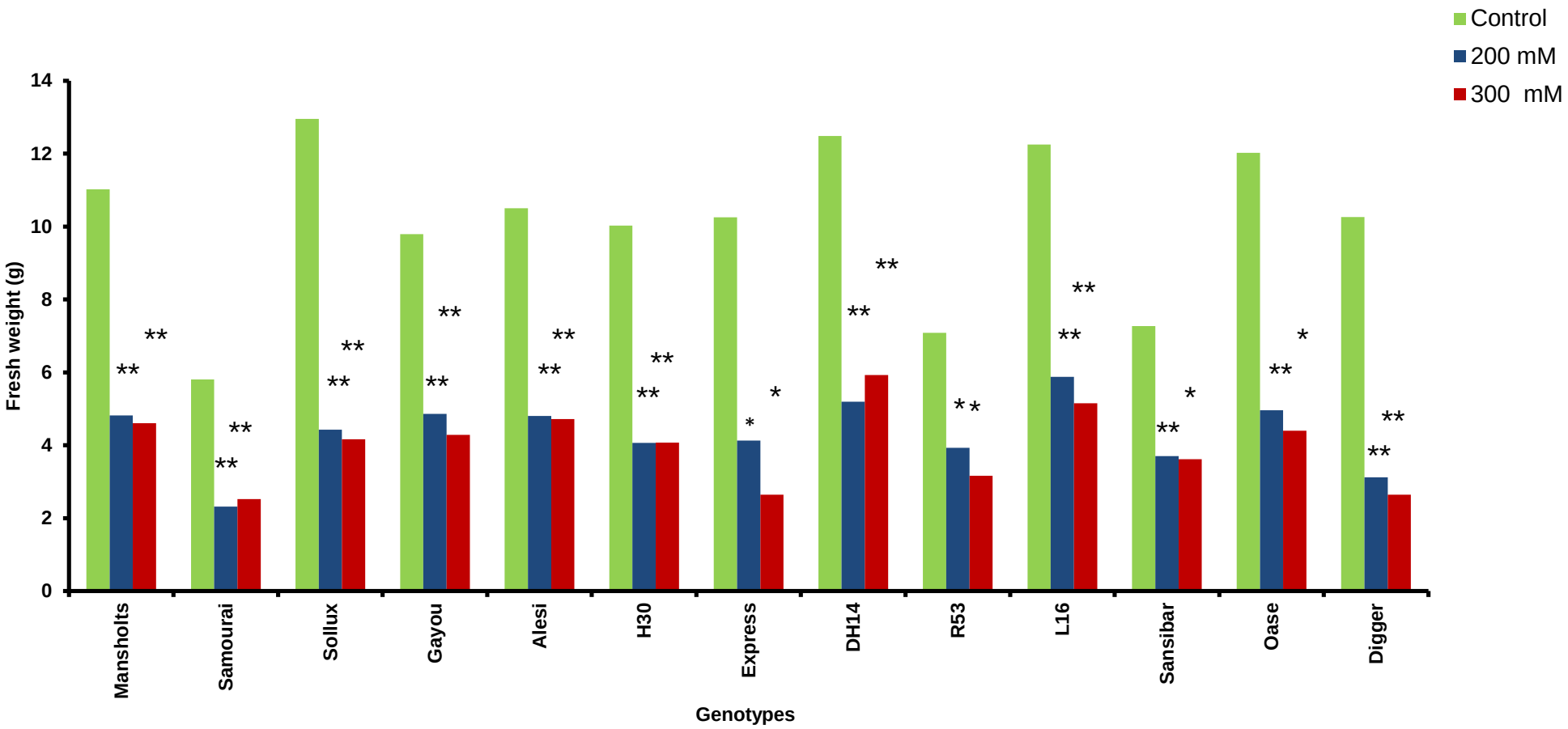


Figure II-1: Total plant fresh weight (g) mean values and significance levels of the 13 parental lines under control and salt stress

** Significant at *P* = 0.01; * significant at *P* = 0.05, + significant at *P* = 0.1 and ns = non-significant. The significance test was done by simple ANOVA using the software Plabstat (Utz 2003).

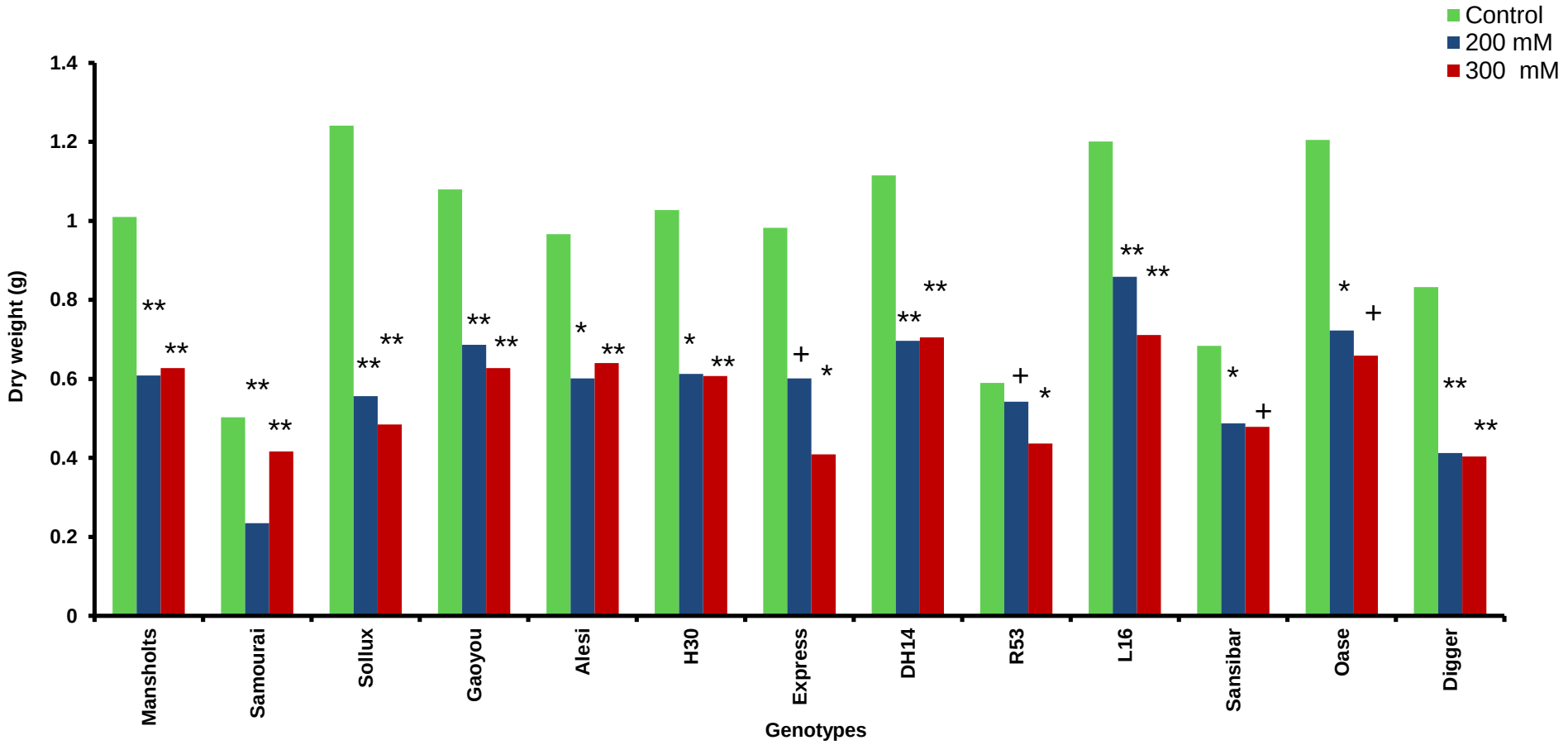


Figure II-2: Total plant dry weight (g) and significance levels of the 13 parental lines under control and salt stress

** Significant at *P* = 0.01; * significant at *P* = 0.05, + significant at *P* = 0.1 and ns = non-significant.

2.3 Experiment 2: Salinity effect on the selected six genotypes; Mansholts, Samourai, Sollux, Gaoyou, Alesi and H30.

2.3.1 Materials and methods

The six selected genotypes**:** Mansholts, Samourai, Sollux, Gaoyou, Alesi and H30 were tested under both control and the salt treatment of 200 mM NaCl. The soil contents, pot size, pots and randomization were the same a mentioned above. Experiment one (2.2): In experiment one, some leaves became chlorotic earlier; therefore, to avoid nutrient deficiency the control treatment and saline solution were both supplied with Hakaphos blue (COMPO, Netherlands).

At day 15, the salt treatment was started with 100 mM NaCl supplemented with 0.5 g/l Hakaphos in the saline solution. At day 17, the saline solution concentration was increased to 150 mM NaCl enriched with 1 g/l Hakaphos. Finally, at day 19 the final concentration of 200 mM NaCl was reached. From day 19 until the end of the experiment the stressed plants were watered with the final concentration on alternative days; one day with water only and the second day with the saline solution. The control plants were always watered with tap water enriched with 1 g/l Hakaphos.

The chlorophyll content was measured with a SPAD-meter Minolta 502 (Osaka, Japan). The measurement was made one week from the beginning of salt treatment. The measurements were scored for two different plant parts: the 1^{st} and the 2^{nd} leaves, and the 3^{rd} and the 4^{th} leaves. The plants were harvested two weeks from the beginning of the salt treatment. The shoot system was separated into 1^{st} and 2^{nd} leaves, 3^{rd} and 4^{th} leaves, stem and rest. Fresh weight was determined immediately after harvesting. For DW estimation, the plant parts were dried for 72 h at 60°C.

2.3.2 Sodium and potassium content analysis

For sodium and potassium concentration measurement, the dried samples were pulverized, using a coffee grinder (KRUPS 75, Germany). One day before digestion the samples were dried overnight at 100 °C. 300 mg from each sample were placed in the microwave system MLS-MEGA II (Leutkirch, Germany). Four ml 65% HNO_3 and two ml H_2O_2 30% (Roth, Germany) were added for each sample. The samples were placed in the microwave system at 200°C for 55 minutes under 15 atmospheric pressure (atm) and cooled down for 20 minutes. After digestion, the samples were diluted up to 25 ml using Seralpur water (deionized and filtered water). The samples were further diluted at 1:10 (0.5 ml plant material extract + 4.5 ml Seralpur water). The Na^+ and K^+ concentration was measured using the flame photometer Eppendorf, Elex 6361 (Hamburg, Germany). The flame photometer was calibrated along with ten samples using two calibration standards for both elements. The low standard was 0 mg/ l Na^+ and 0 mg/ l K^+ and the high standard was 100 mg/ l Na^+ and 100 mg/ l K^+. The Na^+ and K^+ contents were calculated as mg g^{-1} DW and the Na^+/ K^+ ratio was calculated.

2.3.3 Results

The salt treatment caused a significant reduction in the total plant FW and the total plant DW of the tested genotypes (Figures 3 and 4). The parental lines tested showed different interaction with salinity. The reductions in the entire plant FW were 60% for

Samourai and 50% for Mansholts, respectively. The DW reductions were 53% and 40% for Samourai and Mansholts, respectively (Table 1, and Figures 3 and 4). In the cases of the Sollux and Gaoyou lines, the reductions in plant FW were 60% and 51%, respectively. Also the reductions in the total DW of Sollux and Gaoyou were 55% and 37%, respectively (Table 2, and Figures 3 and 4). The reductions in FW were 55% and 60%, for Alesi and H30, respectively. The DW reductions were 39% and 40% for Alesi and H30, respectively. The biomass yield was higher in case of Mansholts, Samourai, Sollux, and Gaoyou lines compared with Alesi and H30. The variations between Alesi and H30 were non-significant (Table 3, and Figures 3 and 4). All parental lines showed a significant variation in the other parameters, such as the Na^+ content of the 1st and the 2nd leaves and also the total plant Na^+ content.

2.3.4 Conclusion

The flooding system is applicable and efficient. A number of parameters, including the FW, DW, Na^+ content of the total plant and the Na^+ content of 1st + 2nd leaves were relevant parameters to differentiate among the considered genotypes. As these parameters were suitable for phenotyping, the other parameters were not included in the next evaluation experiments. Because of the non-significant genotypic variation between Alesi and H30, they were not included in the further evaluation experiment.

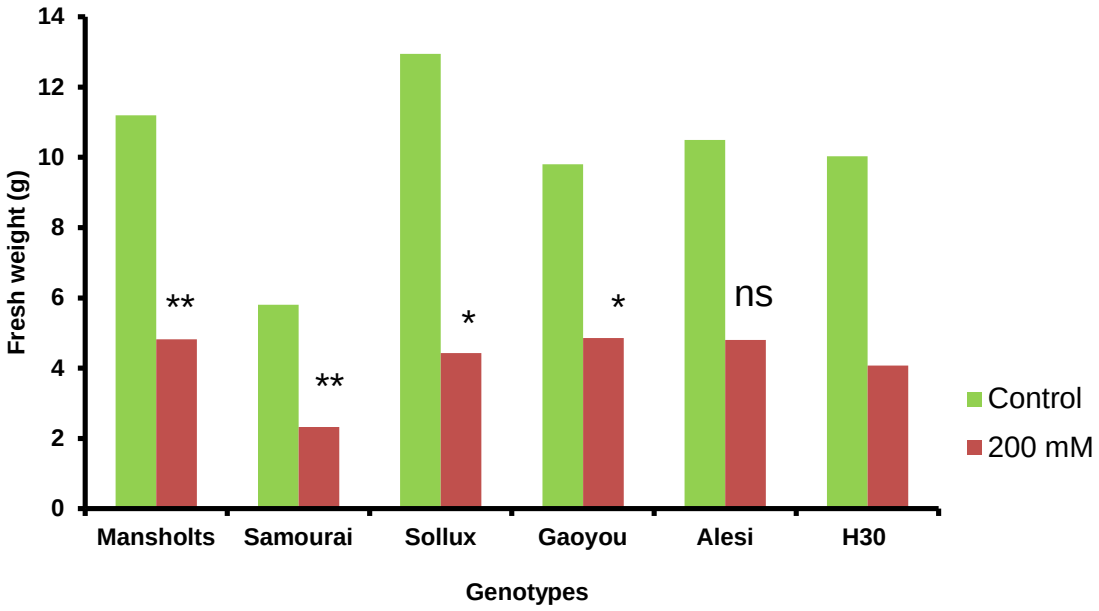


Figure II-3: Total plant fresh weight (g) and signifcance levels of the six genotypes: Mansholts, Samourai, Sollux, Gaoyou, Alesi and H30 under control and salt stress

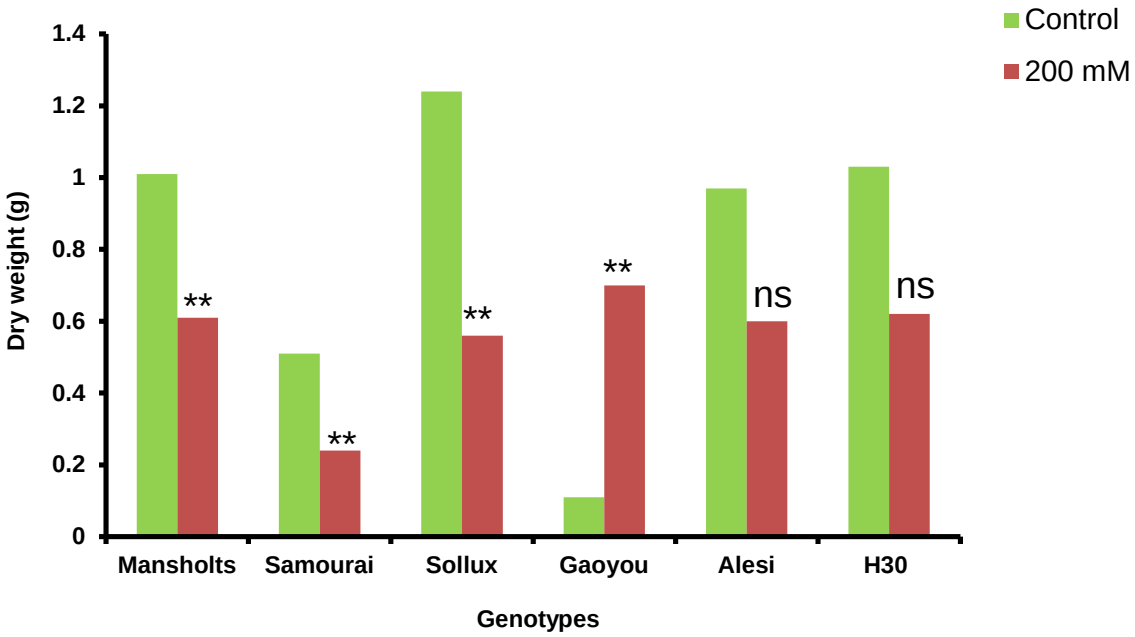


Figure II-4: Total plant dry weight (g) mean values and signifcance levels of the six genotypes: Mansholts, Samourai, Sollux, Goyou, Alesi and H30 under control and salt stress

** Significant at *P* = 0.01; * significant at *P* = 0.05, + significant at *P* = 0.1 and ns = non-significant.

Table II-1: Experiment 2: The mean values of fresh weight (g), dry weight (g), SPAD values, sodium content Na^+ mg/ g DM and potassium content K^+ mg/ g DM of the different parts in Mansholts and Samourai under control and salt stress (200 mM NaCl)

Genotypes	Mansholts	Mansholts	Samourai	Samourai
Traits / treatments	control	salt	control	salt
FW 1+2 (g)	2.50	2.36	1.94	1.21
FW 3+4 (g)	3.98	2.10	2.34	0.69
FW rest (g)	3.41	0.17	0.97	0.25
FW stem (g)	0.92	0.2	0.58	0.18
FW total Plant (g)	11.02	4.82	5.80	2.32
DW 1+2 (g)	0.21	0.26	0.17	0.12
DW 3+4 (g)	0.35	0.29	0.19	0.06
DW rest (g)	0.34	0.03	0.10	0.04
DW stem (g)	0.10	0.04	0.06	0.04
DW total Plant (g)	1.01	0.61	0.51	0.24
SPAD1 1+2	25.17	29.73	29.23	33.60
SPAD1 3+4	26.85	31.45	30.90	34.42
SPAD 2 1+2	24.85	35.28	29.52	34.38
SPAD 2 3+4	30.88	41.92	35.02	37.28
Na^+ 1+2 (Na^+ mg/ g DM)	3.83	16.40	0.97	15.79
Na^+ 3+4 (Na^+ mg/ g DM)	5.07	16.52	1.44	10.02
Na^+ rest (Na^+ mg/ g DM)	3.65	5.90	1.09	5.43
Na^+ stem (Na^+ mg/ g DM)	4.16	5.08	1.33	4.68
Na^+ total Plant (Na^+ mg/ g DM)	2.54	15.53	1.20	12.59
K^+ 1+2 (K^+ mg/ g DM)	20.98	6.61	12.39	11.80
K^+ 3+4 (K^+ mg/ g DM)	16.49	7.49	18.12	6.38
K^+ rest (K^+ mg/ g DM)	16.31	3.18	11.69	4.06
K^+ stem (K^+ mg/ g DM)	15.96	3.60	10.76	4.69
K^+ Total Plant (mg K^+ mg/ g DM)	17.41	6.80	14.26	9.23

Table II-2: Experiment 2: The mean values of fresh weight (g), dry weight (g), SPAD values, sodium content Na^+ mg/ g DM and potassium content K^+ mg/ g DM of the different parts in Sollux and Gaoyou under control and salt stress (200 mM NaCl)

Genotypes	Sollux	Sollux	Gaoyou	Gaoyou
Traits / treatments	control	salt	control	salt
FW 1+2 (g)	4.93	1.90	3.44	1.88
FW 3+4 (g)	5.19	2.02	3.67	2.35
FW rest (g)	1.23	0.17	1.01	0.24
FW stem (g)	1.42	0.34	1.21	0.41
FW total Plant (g)	12.95	4.43	9.80	4.86
DW 1+2 (g)	0.41	0.21	0.35	0.25
DW 3+4 (g)	0.52	0.27	0.39	0.34
DW rest (g)	0.14	0.04	0.17	0.05
DW stem (g)	0.13	0.05	0.11	0.07
DW total Plant (g)	1.24	0.56	1.08	0.69
SPAD1 1+2	26.95	35.47	27.88	32.58
SPAD1 3+4	28.47	34.92	27.25	35.62
SPAD 2 1+2	28.40	40.10	30.17	38.62
SPAD 2 3+4	34.35	44.80	35.25	40.62
Na^+ 1+2 (Na^+ mg/ g DM)	1.38	14.95	4.31	17.69
Na^+ 3+4 (Na^+ mg/ g DM)	1.36	17.15	3.40	14.59
Na^+ rest (Na^+ mg/ g DM)	1.20	3.09	2.51	4.05
Na^+ stem (Na^+ mg/ g DM)	2.33	8.64	4.57	10.67
Na^+ Total Plant (Na^+ mg/ g DM)	1.46	14.39	3.43	15.23
K^+ 1+2 (K^+ mg/ g DM)	22.77	7.60	18.65	12.04
K^+ 3+4 (K^+ mg/ g DM)	17.10	9.60	15.72	9.29
K^+ rest (K^+ mg/ g DM)	14.24	3.09	15.68	4.65
K^+ stem (K^+ mg/ g DM)	23.89	5.19	17.42	6.59
K^+ total Plant (K^+ mg/ g DM)	19.50	7.95	17.73	9.90

Table II-3: Experiment 2: The mean values of fresh weight (g), dry weight (g), SPAD values, sodium content Na^+ mg/ g DM and potassium content K^+ mg/ g DM of the different parts in Alesi and H30 under control and salt stress (200 mM NaCl)

Genotypes	Alesi	Alesi	H30	H30
Traits / treatments	control	salt	control	salt
FW 1+2 (g)	2.35	1.67	2.47	1.27
FW 3+4 (g)	5.73	2.69	4.97	2.24
FW rest (g)	1.21	0.15	2.07	0.39
FW stem (g)	1.24	0.31	0.52	0.19
FW total Plant (g)	10.51	4.81	10.03	4.07
DW 1+2 (g)	0.20	0.17	0.21	0.16
DW 3+4 (g)	0.52	0.37	0.51	0.35
DW rest (g)	0.13	0.03	0.25	0.08
DW stem (g)	0.13	0.05	0.07	0.04
DW total Plant (g)	0.97	0.60	1.03	0.62
SPAD1 1+2	28.50	30.67	26.35	32.00
SPAD1 3+4	28.65	34.25	28.30	34.28
SPAD 2 1+2	29.25	35.97	26.35	35.75
SPAD 2 3+4	32.97	43.97	40.00	45.30
Na^+ 1+2 (Na^+ mg/ g DM)	2.59	10.60	2.84	12.98
Na^+ 3+4 (Na^+ mg/ g DM)	3.07	16.47	2.48	11.47
Na^+ rest (Na^+ mg/ g DM)	2.07	2.88	1.50	5.89
Na^+ stem (Na^+ mg/ g DM)	3.15	6.87	2.15	3.35
Na^+ total Plant (Na^+ mg/ g DM)	2.83	14.14	2.41	10.68
K^+ 1+2 (K^+ mg/ g DM)	19.70	8.50	20.19	12.75
K^+ 3+4 (K^+ mg/ g DM)	17.52	9.22	19.10	11.47
K^+ rest (K^+ mg/ g DM)	15.05	1.93	16.84	8.50
K^+ stem (K^+ mg/ g DM)	21.55	6.40	12.61	3.23
K^+ total Plant (mg K^+ mg/ g DM)	18.18	8.95	18.22	10.95

2.4 Experiment 3: Testing the performance of Mansholts × Samourai and Sollux × Gaoyou under 200 mM NaCl

2.4.1 Material and Methods

The experiment was conducted in the greenhouse from the 23th of July until the 27th of August, 2012. The methods and calculations were the same as the previously mentioned protocol for Experiment 1 (2.2). Two weeks after beginning the 200 mM NaCl salt treatment, the plants were harvested and separated into 1st and 2nd leaves, and the remaining shoot system was labeled as “rest”.

2.4.2 Results

There was a significant reduction in the total plant FW and the total plant FW of the tested genotypes. The reductions in FW for Samourai and Mansholts were 62% and 53%, respectively. The reductions in DW for Samourai and Mansholts were 47% and 37%, respectively (Table 4). In case of, Sollux and Gaoyou lines, the respective reductions in FW were 66 % and 51%, and were 52% and 31% for the DW (Table 6, and Figures 5 and 6).

2.4.3 Conclusion

There was a significant difference between Mansholts and Samourai and between Sollux and Gaoyou, but the performance of both couples was similar (Figures 3 and 4). Nevertheless, the parental lines Mansholts and Samourai were considered to be suitable parental lines based on the molecular markers set and the quality of the linkage map.

2.5 General conclusion

The difference between Mansholts and Samourai was similar to that between Sollux and Gaoyou. However, the DH population derived from the Sollux and Gaoyou parental lines includes 200 DH lines, while that derived from the Mansholts and Samourai parental lines includes 150 DH lines. After discussion with Dr. Ecke, Department of Crop Sciences, Georg-August-Universität Göttingen, we decided to continue with the DH population that was developed from the Mansholts and Samourai parental lines. The selection was based on the quality of the genotypic markers data (linkage map). The linkage map for the Mansholts and Samourai population is preferable because it is better covered by markers than that of Sollux and Gaoyou populations. This is because of the high percentage of markers that were not mapped for Sollux and Gaoyou population. Additionally, one of the linkage groups was lost for the Sollux and Gaoyou population. We found the evaluation parameters, “Na^+ content of the 1st + 2nd leaves” and “Na^+ content of the total plant” to be relevant in testing for salt tolerance. However, so as to reduce labor during the evaluation of the mapping population, measurement of the Na^+ content of the 1st + 2nd leaves was omitted.

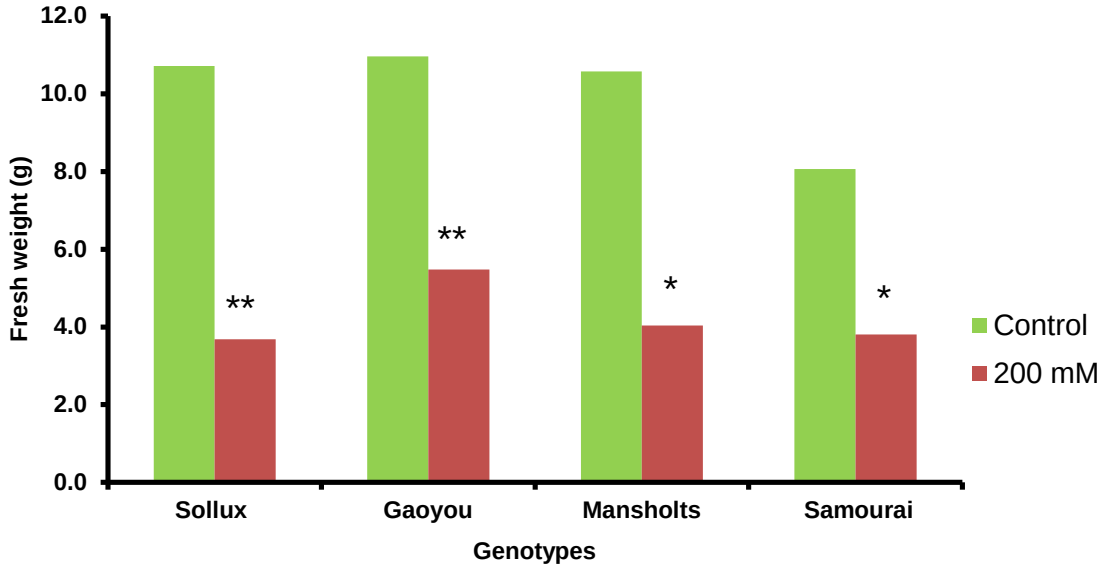


Figure II-5: Total plant fresh weight (g) mean values and signifcance levels of Mansholts, Samourai, Sollux and Gaoyou under control and salt stress

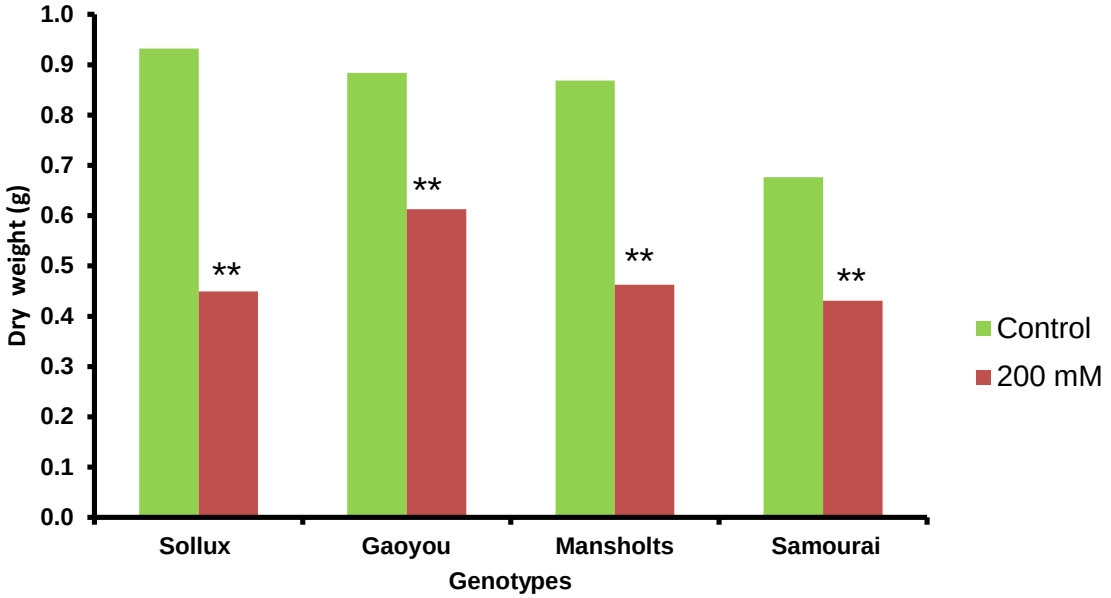


Figure II-6: Total plant dry weight (g) mean values and significance levels of Mansholts, Samourai, Sollux and Gaoyou under control and salt

** Significant at *P* = 0.01; * significant at *P* = 0.05, + significant at *P* = 0.1 and ns = non-significant.

Table II-4: Experiment 3: The mean values of fresh weight, dry weight, sodium content Na^+ mg/ g DM and potassium content K^+ mg/ g DM of the different parts in Mansholts and Samourai under control and salt stress (200 mM NaCl)

Genotypes	Samourai	Samourai	Mansholts	Mansholts
Traits / treatments	control	salt	control	salt
FW 1+2 (g)	1.40	1.27	0.99	1.19
FW rest (g)	9.18	2.77	7.07	2.62
FW total Plant (g)	10.58	4.04	8.06	3.81
DW 1+2 (g)	0.09	0.14	0.07	0.14
DW rest (g)	0.78	0.33	0.61	0.30
DW total Plant (g)	0.87	0.46	0.68	0.43
Na^+ 1+2 (Na^+ mg/ g DM)	4.47	20.34	5.55	24.06
Na^+ rest (Na^+ mg/ g DM)	3.11	18.91	3.94	20.99
Na^+ total Plant (Na^+ mg/ g DM)	3.24	19.30	4.11	21.85
K^+ 1+2 (K^+ mg/ g DM)	12.99	8.58	11.47	7.6
K^+ rest (K^+ mg/ g DM)	13.25	6.91	12.32	6.40
K^+ total Plant (K^+ mg/ g DM)	13.22	7.38	12.23	6.79

Table II-5: Experiment 3: The mean values of fresh weight (g), dry weight (g), sodium content Na^+ mg/ g DM and potassium content K^+ mg/ g DM of the different parts in Sollux and Gaoyou under control and salt stress (200 mM NaCl)

Genotypes	Samourai	Samourai	Mansholts	Mansholts
Traits / treatments	control	salt	control	salt
FW 1+2 (g)	2.52	1.42	2.53	2.45
FW rest (g)	8.19	2.26	8.60	3.02
FW total Plant (g)	10.71	3.68	11.13	5.49
DW 1+2 (g)	0.18	0.17	0.18	0.27
DW rest (g)	0.76	0.29	0.71	0.36
DW total Plant (g)	0.93	0.45	0.89	0.62
Na^+ 1+2 (Na^+ mg/ g DM)	3.75	16.15	5.75	18.11
Na^+ rest (Na^+ mg/ g DM)	2.64	13.69	4.13	16.07
Na^+ total Plant (Na^+ mg/ g DM)	2.85	14.60	4.45	16.93
K^+ 1+2 (K^+ mg/ g DM)	12.49	8.18	10.62	7.38
K^+ rest (K^+ mg/ g DM)	12.40	6.11	12.24	6.47
K^+ total Plant (K^+ mg/ g DM)	12.41	6.88	11.90	6.86

Chapter III

Mapping QTL for salt tolerance at seed germination in *Brassica napus* and *Brassica oleracea* doubled-haploid populations

3.1 Introduction

Salinity is one of the most significant abiotic stresses that reduce crop productivity (Munns 1993). Timely germination is critical for the plant to commence a new life cycle. Seed germination starts when the dry seeds absorb the water and is completed when the radicle protrudes from the seed coat (Bewley 1997a). Physiologically, seed germination encompasses three consecutive phases; (I) rapid water uptake, (II) metabolic activity of enzymes to catalyze the utilization of stored nutrients and (III) embryo enlargement and radical protrusion (Bewley 1997a). As a consequence of imbibition, protein synthesis and DNA transcription are reactivated. The cell wall-weakening enzymes facilitate the penetration of the radicle through the endosperm and seed coat, and the stored energy sources are remobilized to ensure a fast growth of the emerging seedling (Nonogaki 2006). High concentrations of salt impair seed germination and crop establishment (Flowers 1991). The loss of optimum plant germination causes a reduction in plant density, which may result in yield reduction. The germination rate and seedling establishment of *B. napus* is delayed and reduced by salinity (Zheng et al. 1998).

QTL and genes related to salt tolerance at seed germination

QTL associated with salt tolerance during seed germination have been detected in many plants such as tomato (Foolad and Jones 1993), barley (Mano and Takeda 1997), wheat (Ma et al. 2007) rice (Wang et al. 2011) and *Arabidopsis* (Quesada et al. 2002; Ren et al. 2010; Vallejo et al. 2010; Joosen et al. 2012). For salt tolerance, Ren et al. (2010) identified a premature stop codon in the *RAS1* (*RAS1*; At1g09950) gene that acts as a negative regulator of salt tolerance during seed germination and early seedling growth by enhancing ABA sensitivity in *Arabidopsis.* In *Arabidopsis*, one major QTL has been detected that controls both the germination percentage and the germination rate. In addition, one of the genes that were identified in this QTL interval is a nicotinamidase gene (*NIC2*, At5g23230) (Joosen et al. 2012). The function of this gene is to repair DNA prior to germination. The mutant of this gene causes retarded germination and impaired germination potential (Hunt et al. 2007). Another strong QTL harboring two potential candidate genes involved in the osmotic stress pathway (NHX1; AT5G27150 and H^+ ATPase; AT5G08690) was localized on the top of chromosome 5 in *Arabidopsis* (Joosen et al. 2012).

3.2 The objectives of this chapter are to

1. assess the variation in germination under salt stress.
2. Identify the QTL that controls seed germination variation under control and salt stress conditions in *Brassica napus* and *Brassica oleracea* DH populations.

3.3 Materials and methods

3.3.1 Plant materials

Three doubled-haploid (DH) mapping populations were investigated; two populations of *B. napus* and one of *B. oleracea.* The *B. napus* DH population consisted of 138 DH lines derived from a cross between Alesi (winter oilseed rape cultivar) and H30 (resynthesized line). Alesi has 00 seed quality, a low glucosinolate content and zero erucic acid in the seeds, whereas H30 has ++ seed quality with high erucic acid and high glucosinolate content in the seeds. Doubled-haploid lines of this population were provided by KWS SAAT AG, Einbeck, Germany.

The second mapping population consisted of 138 DH lines developed from a cross between two DH lines DH5.1 and DH5.2 from variety Mansholts Hamburger Raps and one DH line from the cultivar Samourai DH11.4. Mansholts Hamburger Raps is an old cultivar with ++ seed quality and Samourai is a modern French cultivar with 00 seed quality (Uzunova et al. 1995).

The third population is a *B. oleracea* Bo1TBDH population, which consisted of 145 DH lines derived from a cross between DH rapid cycling cabbage line TO1000DH3 and DH broccoli line Early Big. The population was developed as a *B. oleracea* reference population. The parental line TO1000DH3 is the reference genome for the *B. oleracea* sequencing project (Iñiguez-Luy et al. 2009). The lines of this population were provided by Graham Teakle, Warwick Crop Centre, The University of Warwick, Wellesbourne, Warwick.

3.3.2 Germination experiment

For germination, ten seeds per genotype were placed in 9-cm-diameter Petri dishes on Whatman No1 paper moistened, in the case of the controls, with 5 ml of tap water. For the salinity experiments the set-up was similar, except the paper was moistened with 5 ml of 200 mM NaCl solution for the *B. napus* Alesi and H30, and Mansholts and Samourai populations, and with five ml of 100 mM NaCl solution for the *B. oleracea* (Bo1TBDH) population, respectively. The Petri dishes were placed in an incubator Rubarth Apparate Gmbh (Hannover, Germany) at 20°C under dark conditions. All DH lines and parental lines were tested in three replicates and in a complete randomized block design. Germination was scored at 24 hour intervals for up to eight days. The seeds were considered germinated when a complete radicle protrusion appeared through the seed coat (Bewley 1997a).

Three germination parameters were assessed; germination percentage (G%), germination pace (GP) and the Salt Tolerance Index (STI).

$$G\% = \frac{n}{N} \text{x } 100$$

Where n is the number of germinated seeds at the end of the experiment and N is the number of total sown seeds.

$$GP = \frac{N}{\Sigma\ (n \times g)} \times 100$$

Where N is the total number of germinated seeds at the end of the experiment, n is the number of germinated seeds on day g.

The salt tolerance index (STI) was calculated for G% and GP, according to Cano et al. (1998).

$$G\%\ STI = \frac{G\%\ \text{under salt stress}}{G\%\ \text{under control}} \times 100$$

$$GP\ STI = \frac{GP\ \text{under salt stress}}{GP\ \text{under control}} \times 100$$

3.3.3 Statistics

The single values for each genotype of the three replicates were used for the analysis of variance, and the heritabilities were analyzed using PlabSTAT 3.0 software (Utz, 2003) according to the following model:

$$Y_{ij} = \mu + g_i + r_j + (gr)ij$$

where Y_{ij} is the observation of genotype i in replicate j, μ is the general mean, g_i is the effect of genotype i (for i=1…, n), r_j is the replicate effect and (g r)ij is the experimental error.

3.3.4 Linkage maps

To match with the QTL mapping software PlabMQTL requirements, we employ the Haldane's mapping function as default. The recombination frequencies between the markers on the full maps and the framework maps of all populations were transformed into mapping distances in centiMorgans (cM) with the Haldane's mapping function (Haldane 1919). In all populations: *B. napus* Alesi and H30, *B. napus* Mansholts and Samourai, and *B. oleracea* Bo1TBDH, the linkage groups (LGs) were named according to the Multinational *Brassica* genome project (MBGP), http://www.brassica.info/. Date of visiting January 15, 2014.

The linkage map of *B. napus* mapping population (Alesi and H30)

The marker data for the DH population Alesi and H30 were developed by KWS SAAT AG, Einbeck, Germany and Sebastian Miersch, Department of Crop Sciences, Georg-August-Universität Göttingen. The full map was developed by Sebastian Miersch based on 139 DH lines using 438 markers: 390 single nucleotide polymorphism markers (SNP) and 48 simple sequence repeat markers (SSR). The mapped markers were distributed on 22 linkage groups (LGs). The LG C4 was subdivided into C4a and C4b. Moreover, the LG C5 was subdivided into C5a and C5b, and four markers were mapped on the LG 22. The markers set covered 1483 cM of the *B. napus* genome with an average marker density of one marker per 3.6 cM (Table 1, and Appendix 2).

For QTL mapping, a framework map was developed consisting of 188 markers for QTL mapping (Figure 4). A well-distributed, highly informative set of markers was selected to have one marker every 5 cM to 10 cM wherever possible. The order of the selected markers was verified using MAPMAKER/EXP 3.0 (Lincoln et al. 1993) and the command “ripple” with window six markers and threshold with a LOD score three.

The linkage map of *B. napus* mapping population of Mansholts and Samourai

The full map of Mansholts and Samourai population based on 177 DH lines was an extended version of the map developed by Uzunova et al. (1995). The extended map consisted of 613 markers: 214 Restriction fragment length polymorphism markers (RFLP), 228 Amplified fragment length polymorphism markers (AFLP), 135 SSR, 35 Random amplified polymorphic DNA markers (RAPD), and one phenotypic marker (flower color). The mapped markers were distributed on 21 LGs, whereby the LG C8 was subdivided into C8a and C8b, and four markers were mapped on LG 21. The markers set covered 2180 cM of the *B. napus* genome with an average marker density of one marker per 3.6 cM (Table 2 and Appendix 3). A framework map was developed for QTL mapping as described earlier. The framework map consists of 208 markers for QTL mapping.

The linkage map of *B. oleracea* mapping population Bo1TBDH

The full map was developed by Iñiguez-Luy et al. (2009) based on 155 DH lines. This map consists of 279 markers; 155 RFLP, 122 SSR, one phenotypic marker (flower color), and one ispga PCR marker. The mapped markers were distributed on nine LGs, representing the nine chromosomes *of B. oleracea* according to the international nomenclature. The markers set covered 1012.7 cM of the *B. oleracea* genome with an average marker density of one marker per 3.6 cM (Table 3, and Appendix 4). Based on this map, a framework map of 128 markers was developed as described earlier

3.3.5 QTL analysis protocol

The means of the phenotypic data were analysed with the genetic markers using software QTLNetwork2.1 (Yang et al. 2008) and PlabMQTL (PLAnt-Breeding Meta QTL-analysis) Version 9 (Utz 2011). In the first analysis using QTLNetwork2.1, the significance level of QTL detection was fixed to $P = 0.05$. QTL resulting from this analysis were considered to be “significant QTL”. Additional non-significant QTL were defined as “putative QTL”. The QTL analysis was repeated using PlabMQTL at $P = 0.05$.

PlabMQTL employs multiple regressions to perform composite interval mapping using cofactors. The QTL mapping takes place in two steps. First, the whole genome is scanned over to produce the LOD curves and to show the peaks where the QTL are located by composite interval mapping (CIM). Second, the set of QTL which were produced from the first step were verified by Bayesian information criterion (BIC) in a stepwise regression procedure to identify the most important QTL. To generate LOD scores corresponding to a significance level of $P = 0.05$ the LOD threshold for each

trait was calculated independently using a permutation test with 1000 replications according to Doerge and Churchill (1996) and use of software PlabMQTL.

Putative QTL were identified either in the QTL analysis by use of QTLNetwork2.1 or on the basis of the LOD score curves (PlabMQTL) as QTL that had low LOD scores, which were nevertheless below the significance threshold. The statistical parameters of these putative QTL were obtained by use of PlabMQTL (LOD 2.0).

Table III-1: Linkage group size, number of markers and marker density per linkage group of the full map of *Brassica napus* DH population Alesi × H30

Linkage group	Size (cM)	Marker number	Marker density
A1	82.7	25	3.3
A2	49.8	12	4.1
A3	153.5	36	4.3
A4	28.3	8	3.5
A5	108.6	29	3.7
A6	110.9	32	3.4
A7	74.6	38	1.9
A8	20.5	11	1.9
A9	89.6	18	4.9
A10	66.2	12	5.5
C1	81.3	28	2.9
C2	8.4	4	2.1
C3	91.6	22	4.1
C4a	74.6	15	4.9
C4b	34	4.0	8.5
C5a	60	15	4.0
C5b	16.9	6	2.8
C6	120.7	52	2.3
C7	57.4	22	2.6
C8	40.8	15	2.7
C9	105.4	32	3.3
Lg22	7.3	2	3.6
Total	**1483.1**	**438**	**3.4**

Table III-2: Linkage group size, number of markers and marker density per linkage group of the full map of *Brassica napus* DH population Mansholts × Samourai

Linkage group	Size (cM)	Marker number	Marker density
A1	85.8	30	2.8
A2	147.8	21	7.0
A3	125.4	54	2.4
A4	67.2	20	3.3
A5	161.2	47	3.4
A6	88.3	27	3.2
A7	77.9	35	2.2
A8	78.5	21	3.7

Table 2 continued from page 33

A9	117.0	34	3.4
A10	72.1	39	1.8
C1	126.6	37	3.0
C2	135.1	26	5.1
C3	176.0	53	3.3
C4	154.1	45	3.4
C5	108.6	12	9.0
C6	104.6	30	3.4
C7	110.8	19	5.8
C8a	76.2	22	3.4
C8b	5.3	10	0.5
C9	141.1	27	5.22
Lg21	20.2	4	5.0
Total	**2180**	**613**	**3.6**

Table III-3: Linkage group size, number of markers, and marker density per linkage group of the full map of *Brassica oleracea* DH population Bo1TBDH

Linkage group	Size (cM)	Marker number	Marker density
C1	107.7	37	2.9
C2	119.8	26	4.6
C3	116	43	2.6
C4	117.4	31	3.8
C5	113.7	41	2.8
C6	130	28	4.6
C7	117.5	19	6.1
C8	100.2	22	4.5
C9	82	32	2.5
Total	**1012.7**	**279**	**3.6**

3.4. Results

3.4.1 Traits variations

A large phenotypic variation was found in all populations. The mean, minimum, and maximum germination percentage, germination pace and respective salt tolerance indices determined in the three DH populations under control and salt treatment are summarized in Appendix 1.

In the *B. napus* DH population of Alesi and H30, the difference between parental lines was high for all traits under both control and salt stress, except for G% under control, where both parents gave 100%. For the DH lines under control conditions G% ranged from 90% to 100%. When they were treated with 200 mM NaCl solution, G% varied from 10% to 100% (Figure 1a, b). Germination pace ranged from 0.18 to 0.62 under control conditions, whereas under salt stress there was a clear reduction extending from 0.11 to 0.35 (Figure 1c, d). A significant variation among the DH lines was found under control and salt stress conditions. For G%, the variation under salt stress was higher than under control conditions. Conversely, for GP, the variation among DH lines was lower under salt stress. Heritability as an estimation of the repeatability of the experiment of G% and GP under control was 57% and 85%, respectively. On the other hand, under salt stress it was 74% and 75% for G% and GP, respectively. The mean squares and heritability of G% and GP under both conditions are summarized in Table 4.

The salt tolerance index (STI) for G% varied from 20% to 100% and for GP from 34% to 81%. Significant differences were observed among the DH lines. The heritability of STI was high for G% (h^2 = 85%) and moderate for GP (h^2 = 47%). The variation of G%, STI and GPSTI was wide, whereby some DH lines showed salt tolerance indices higher than the parent, with high STI values and others were lower than the parent, with the lowest STI value(Figure 1e, f).

In the *B. napus* DH population of Mansholts and Samourai, the difference between the parental lines for all traits was non-significant under control conditions as well as under salt stress (Figure 2). In contrast, the DH lines showed wide variation in all traits, particularly under salt stress. The germination percentage varied from 90% to 100% and from 0% to 100% under control conditions and salt stress, respectively (Figure 2a, b). GP varied under control conditions from 0.21 to 0.61 and ranged from 0 to 0.32 under salt stress (Figure 2c, d). The genetic variation and heritability values for the DH lines were higher under salt stress than under control conditions. The heritability was moderate under control with 45% and 62% for G% and GP, respectively, while it was much higher under salt stress, with 95% and 96% for G% and GP, respectively.

The salt tolerance index for G% varied from 20% to 100% and ranged from 20% to 100% for GP. A transgressive variation was observed in both directions among the DH lines. A set of DH lines exceeded the tolerant parent while others showed values lower than the susceptible parent (Figure 2e, f). Significant differences were found between

the DH lines, though the difference between parental lines was not significant. The heritability of STI was high, with 96% and 85% for G% and GP, respectively. Mean squares and heritability of G%, STI and GPSTI are summarized (Table 5).

In the *B. oleracea* Bo1TBDH mapping population, the differences between the parental lines was small and non-significant under both conditions for all traits. In the DH lines, under salt stress the G% ranged from 0% to 100% and from 60% to 100% under control (Figure 3a, b). For GP, the variation under salt stress went from 0.00 to 0.35 and ranged from 0.13 to 0.40 under control (Figure 3c and 3d). These results indicate that a group of DH lines failed to germinate under the applied salt stress. The significant variation and heritability were higher under the salt treatment than under control. The heritability of G% and GP for control was 86% and 74% and was 90% and 74% under salt, respectively (Table 5).

The salt tolerance index ranged from 0% to 100% for G% as well as for GP (Figure 3e, 3f). The heritabilities for G%STI and GPSTI were 83% and 20% respectively. The mean squares for the respective traits and heritability values are summarized (Table 6).

In all populations, under both conditions, there was a transgressive segregation in both sides, except for the G% in the first population of Alesi and H30, where the parental line H30 displayed 100% seed germination. The observed transgressive segregation means that both parental lines in all populations could contribute positively to increasing the respective trait.

** The names of parents are abbreviated

M = Mansholts
S = Samourai
TO = TO1000DH3
EB = Early Big

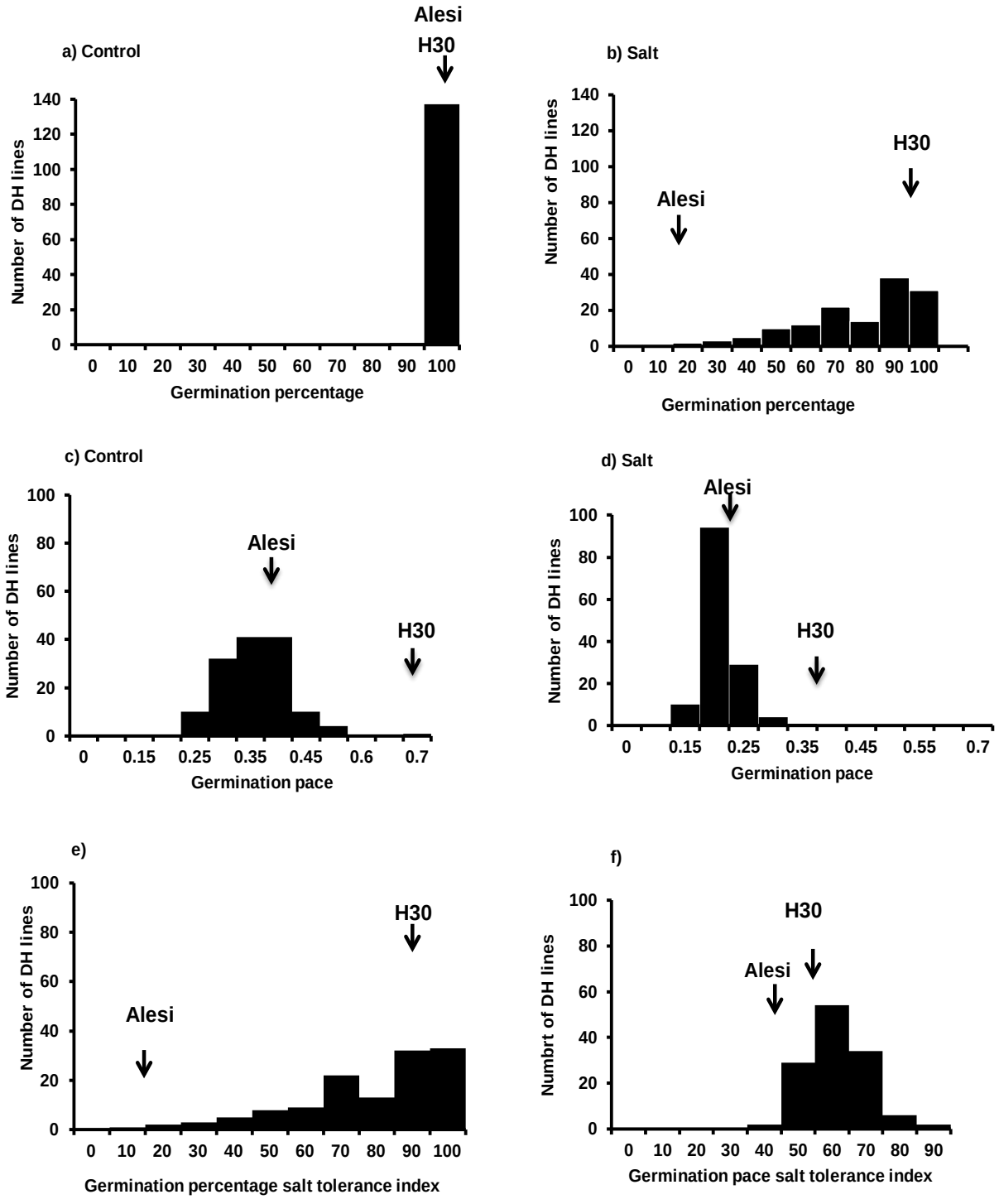


Figure III-1: Frequency distribution of germination parameters under control under salt stress conditions of *Brassica napus* mapping population Alesi × H30; a) Germination percentage (%) under control and b) Germination percentage under salt; c) Germination pace (%) at control and d) Germination pace under salt, e) Salt tolerance index for germination percentage and f) Salt tolerance index for germination pace

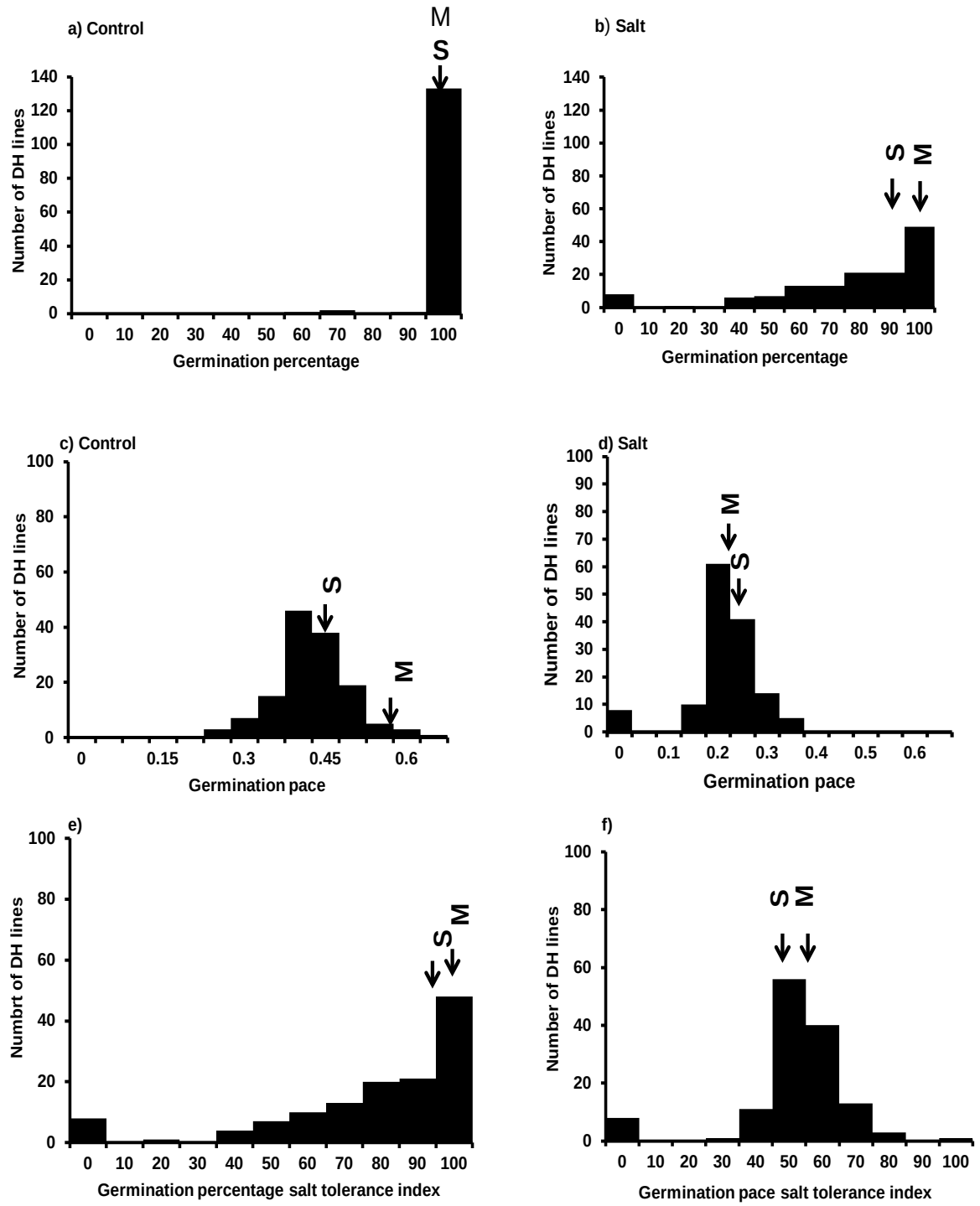


Figure III-2: Frequency distribution of germination parameters under control under salt stress conditions of *Brassica napus* mapping population Mansholts (M) × Samourai (S); a) Germination percentage (%) under control and b) Germination percentage under salt; c) Germination pace (%) under control and b) Germination pace under salt, d) Salt tolerance index for germination percentage and d) Salt tolerance index for germination

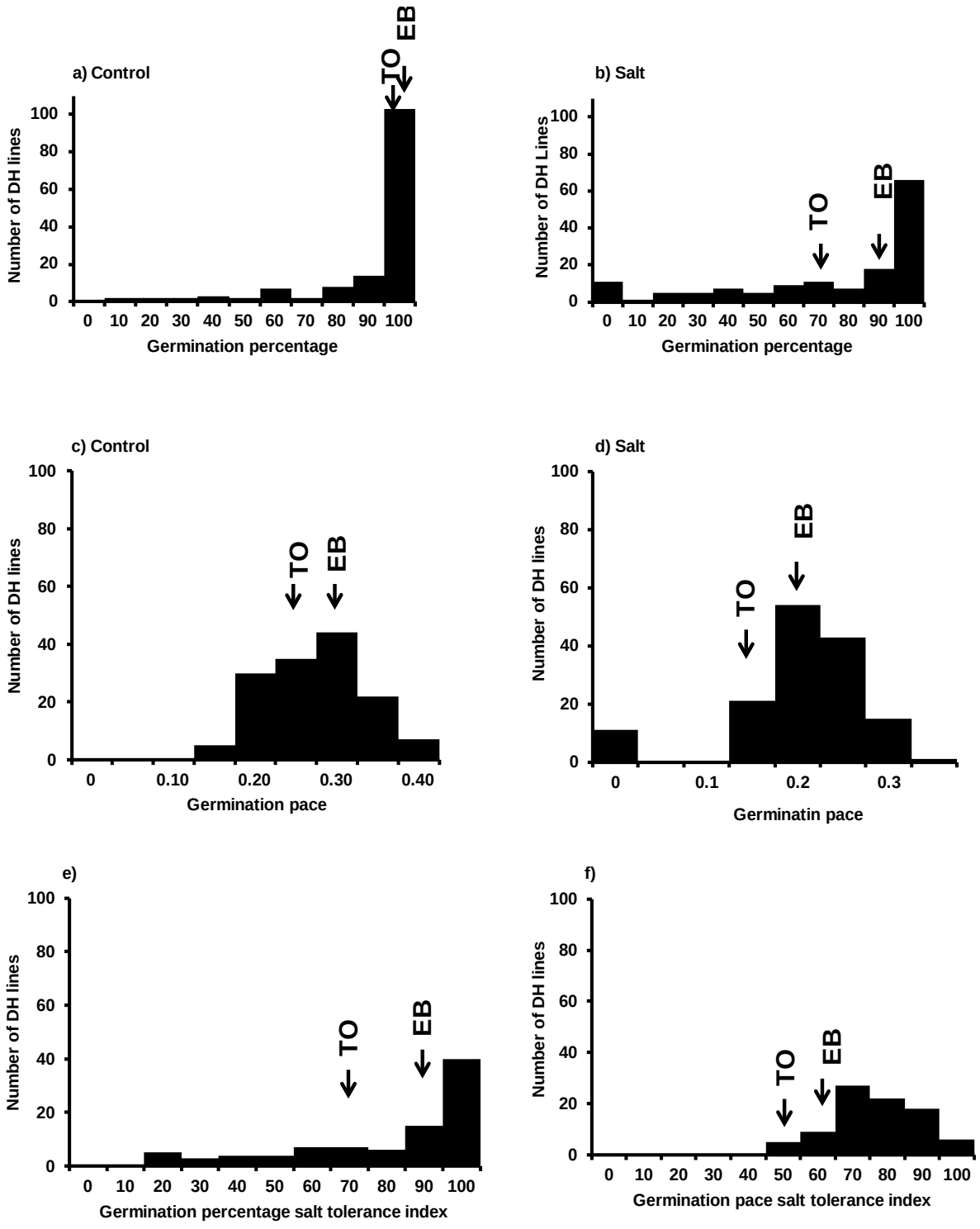


Figure III-3: Frequency distribution of germination parameters under control under salt stress conditions of *Brassica oleracea* mapping population Bo1TBDH; a) Germination percentage (%) under control and b) Germination percentage under salt; c) Germination pace under control and d) Germination pace under salt, e) Salt tolerance index for germination percentage and f) Salt tolerance index for germination pace

Table III-4: Mean squares, respective F tests, and heritabilities estimated from the ANOVA of *Brassica napus* DH population Alesi × H30, 138 DH lines were tested under control conditions and at salt treatment (200mM NaCl)

Sources of variance	DF	Germination Percentage (G %)		Germination Pace (GP)		G% STI	GP STI
		Control	Salt	Control	Salt		
Genotypes (G)	137	6.72**	12.76**	0.93**	0.27**	1269.35**	205.17**
Replicates (R)	2	11.60*	11.27**	2.93**	1.16**	11881.38**	129.13
G × R	274	2.80	18.40	0.23	0.06	189.35	108.50
h^2		57.86	85.62	74.22	75.15	85.08	47.12

Table III-5: Mean squares, respective F tests, and heritabilities estimated from the ANOVA of *Brassica napus* DH population Mansholts × Samourai, 138 DH lines were tested under control conditions and at salt treatment (200mM NaCl)

Sources of variance	DF	Germination Percentage		Germination Pace		G% STI	GP STI
		Control	Salt	Control	Salt		
Genotypes (G)	137	9.0**	20.31**	0.02**	0.01**	2040.01**	670.77**
Replicates (R)	2	2.80	1.60	0.09**	0.00	39.65	637.67**
G × R	274	5.40	11.00	0.07	0.01	112.94	103.29
h^2		44.66	94.57	61.90	95.79	94.46	84.60

Table III-6: Mean squares, respective F tests, and heritabilities estimated from the ANOVA of *Brassica oleracea* DH population Bo1TBDH, 145 DH lines were tested under control conditions and at salt treatment (100mM NaCl)

Sources of variance	DF	Germination Percentage		Germination Pace		G% STI	GP STI
		Control	Salt	Control	Salt		
Genotypes (G)	144	1331.07**	1981.63**	0.01**	0.05**	1736.57**	189.32+
Replicates (R)	2	4520.38**	951.31**	0.24**	0.04**	523.30	4545.14**
G × R	288	184.40	199.83	0.001	0.001	297.87	150.10
h^2		86.15	89.92	73.87	74.43	82.85	19.81

** Significant at $P = 0.01$; * significant at $P = 0.05$ and + significant at $P = 0.1$

3.4.2 Framework maps

For the *B. napus* DH population of Alesi and H30, the framework map comprises 188 markers: 131 SNPs markers and 57 SSR markers were developed as described earlier in Chapter 3 (3.3.4). The mapped markers distributed on 22 LGs, with a marker density of one marker per 4.1 cM. The size of the LGs was unequal. The LG C2 presented the shortest LG with a length of 8.4 cM, while LG A3 was the longest LG with a length of 149.8 cM. The distribution of markers on LGs was uneven, ranging from only two markers on LGs: C4b and LG 22 to 18 on LG C6. Similarly, the marker density differed among the LGs from one marker per 2.8 cM on LG C2 to one marker per 12.4 cM on LG C4b. The set of markers that was mapped on the framework map of this population covered 1444 cM of the *B. napus* genome (Table 7 and Figure 7).

In the *B. napus* DH population of Mansholts and Samourai, the framework map included 208 markers: 121 RFLP, 27 AFLP, 42 SSR, 17 RAPD and 1 phenotypic marker (flower color) was developed as reported before. The selected markers were mapped on 21 LGS, with a marker density of one marker per 10.4 cM. The length of LGs varied from 4.8 cM for LG C8b to 173.6 cM for LG C3. The number of markers mapped per LG varied from three markers on LG C8b to 17 markers on LGs C3 and C4. The marker density ranged from one marker per 1.6 cM on LG C8b to one marker per 15.3 cM on LG C5. This framework map covered 2179 cM of the *B. napus* genome (Table 8 and Figure 8). In *B. oleracea* (Bo1TBDH population), the framework map consisted of 128 markers: 70 RFLP, 56 SSR, 1 phenotypic marker (flower color) and 1 ispga PCR marker was constructed using the protocol described in Chapter 3 (3.3.4). The selected markers were distributed on nine LGs with a marker density of one marker per 3.6 cM. The length of the LGs was longer than 100 cM, but LG C9 was 78.5 cM. All linkage groups were covered with more than 10 markers, except LG C9 with 9 markers. The highest marker density was on LG C1 with one marker per 5.7 cM. The mapped markers on this framework map covered 1000 cM of the *B. oleracea* genome (Table 9 and Figure 9).

Table III-7: Linkage group size, number of markers, and marker density per linkage group of the framework map of *Brassica napus* DH population Alesi × H30

Linkage group	Size (cM)	Marker number	Marker density
A1	78.6	14	5.6
A2	53	5	10.6
A3	149.8	17	8.7
A4	28.2	5	5.6
A5	103	13	7.9
A6	110.6	10	11.0
A7	75.5	14	5.3
A8	20.8	5	4.1
A9	89.5	9	9.9
A10	66.1	8	8.2
C1	79	9	8.7
C2	8.4	3	2.8
C3	89	9	9.8
C4a	74.6	6	12.4
C4b	35.9	3	11.9
C5a	54.2	5	10.8
C5b	14.4	4	3.6
C6	118.8	18	6.6
C7	58.3	10	5.83
C8	41.2	7	5.88
C9	88.5	12	7.3
Lg22	7.3	2	3.6
Total	**1444.7**	**188**	**7.6**

Table III-8: Linkage group size, number of markers, and marker density per linkage group of the framework map of *Brassica napus* DH population Mansholts × Samourai

Linkage group	Size (cM)	Marker number	Marker density
A1	78.5	9	8.7
A2	148.6	12	12.3
A3	131.3	15	8.7
A4	69.3	8	8.6
A5	161.1	11	14.6
A6	87.2	7	12.4
A7	75.5	11	6.8
A8	78.7	8	9.8
A9	119.5	10	10.8
A10	72.6	8	8.8
C1	127.9	10	12.7

Table 8 continued from page 42

C2	132.8	11	12.0
C3	173.6	17	10.2
C4	151.4	16	9.4
C5	110.2	7	15.7
C6	107.1	11	9.7
C7	115.6	10	10.8
C8a	75.1	8	9.3
C8b	4.8	3	1.6
C9	138.1	12	11.5
Lg21	20.2	4	5.0
Total	**2179.2**	**208**	**10.4**

Table III-9: Linkage group size, number of markers, and marker density per linkage group of the framework map of *Brassica oleracea* DH population Bo1TBDH

Linkage group	Size (cM)	Marker number	Marker density
C1	107.7	19	5.7
C2	118.7	13	9.1
C3	115.9	18	6.4
C4	117	17	6.8
C5	114.1	13	8.7
C6	130	15	8.6
C7	116.6	11	10.6
C8	101.8	13	7.8
C9	78.1	9	8.6
Total	**1000**	**128**	7.8

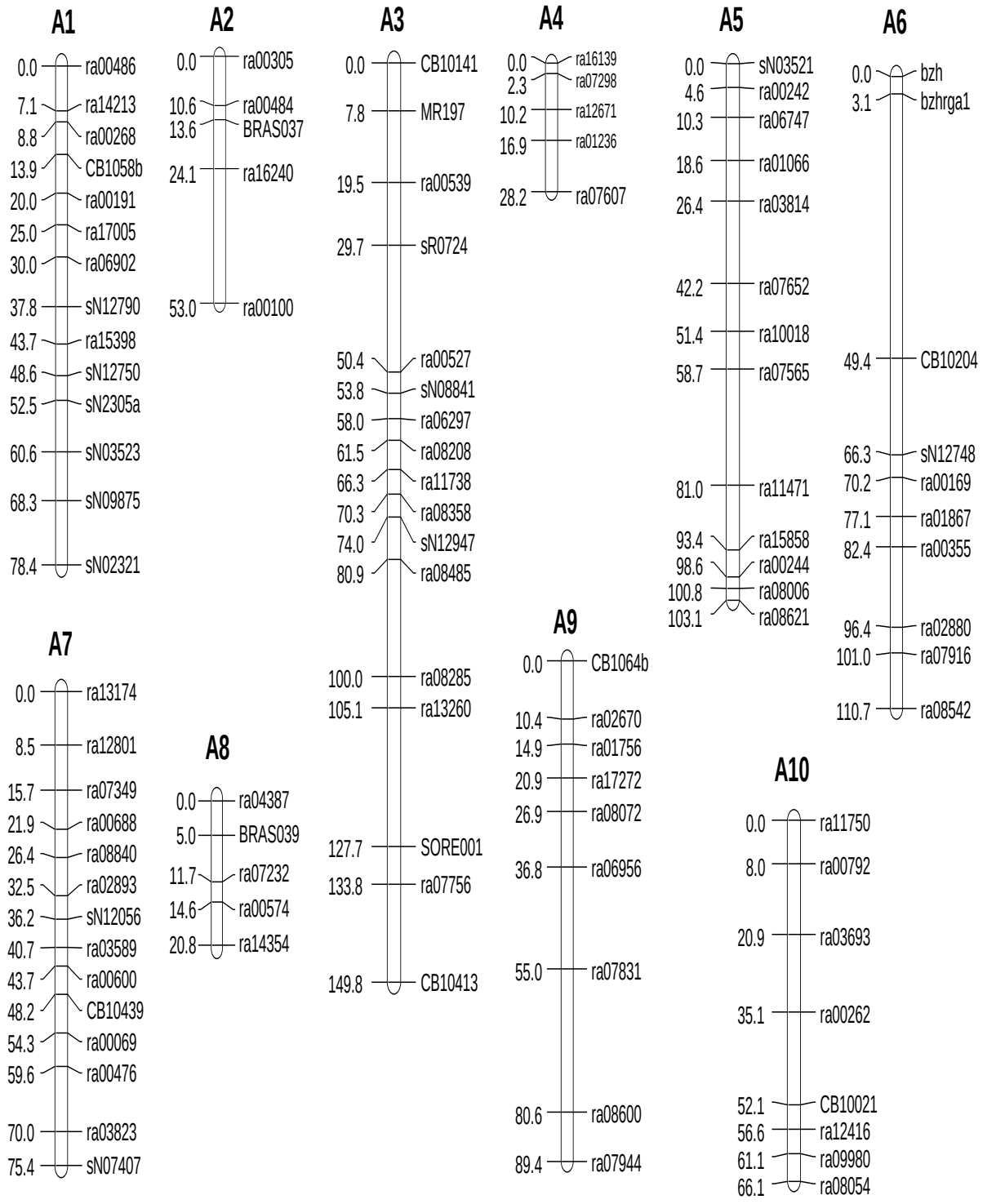
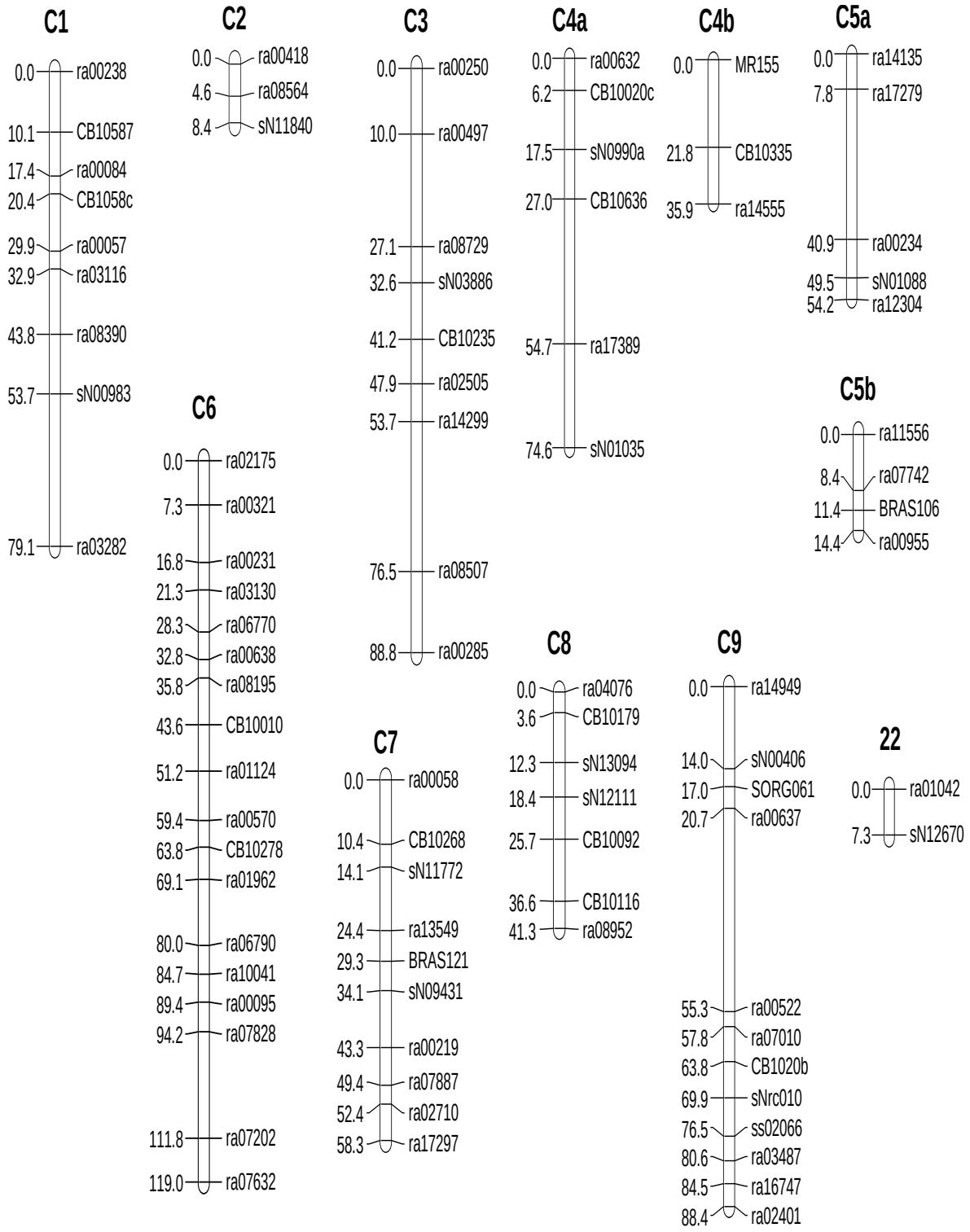


Figure III-4: Framework map of *Brassica napus* mapping population Alesi × H30. The vertical bars are the linkage groups N1-N10 = A1-A10 and N11-N19 = C1-C9 (international nomenclature). Marker locus names and positions (cM) are located to the left and right of the vertical bars, respectively

Figure III-1 continued from page 44



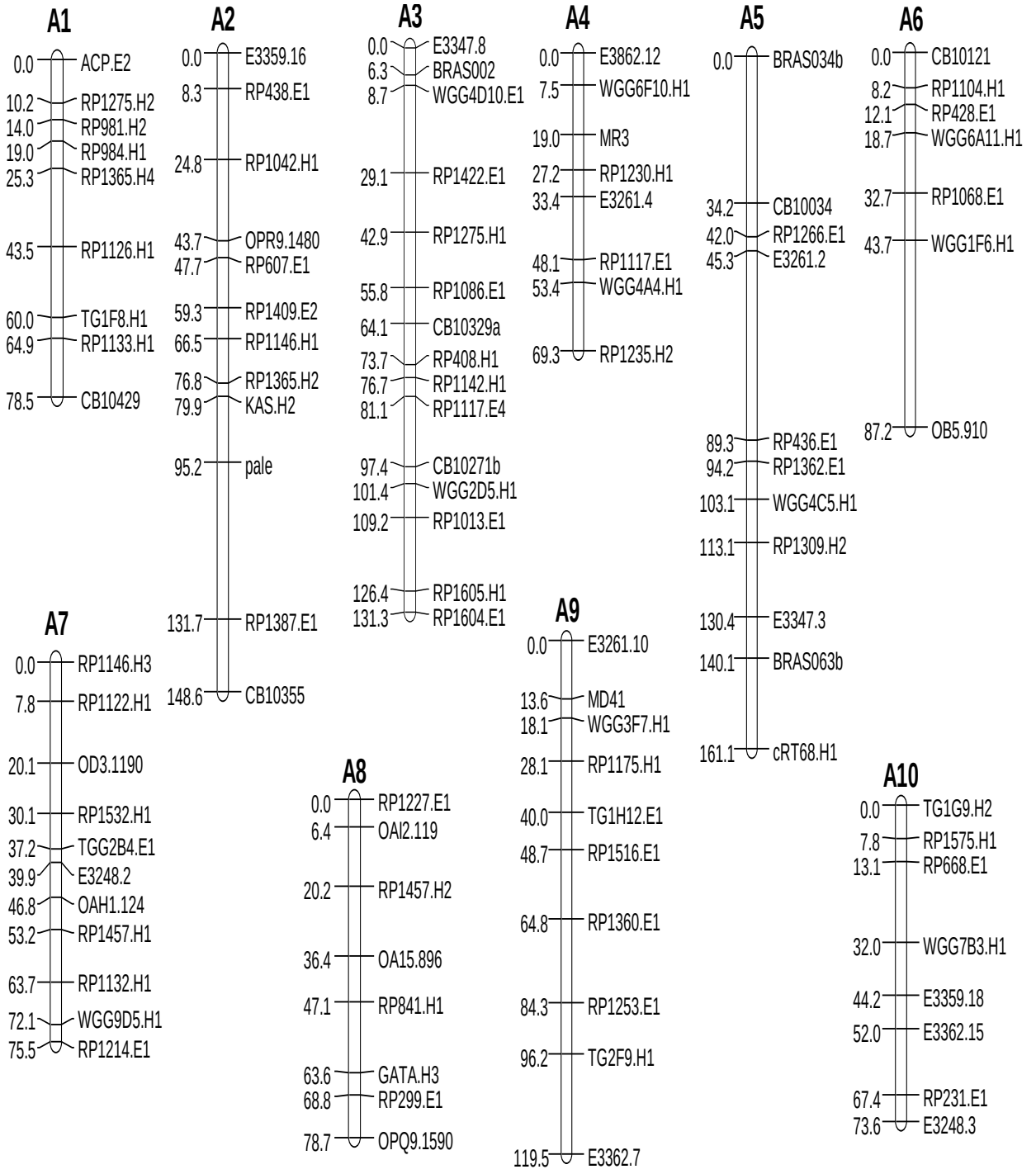
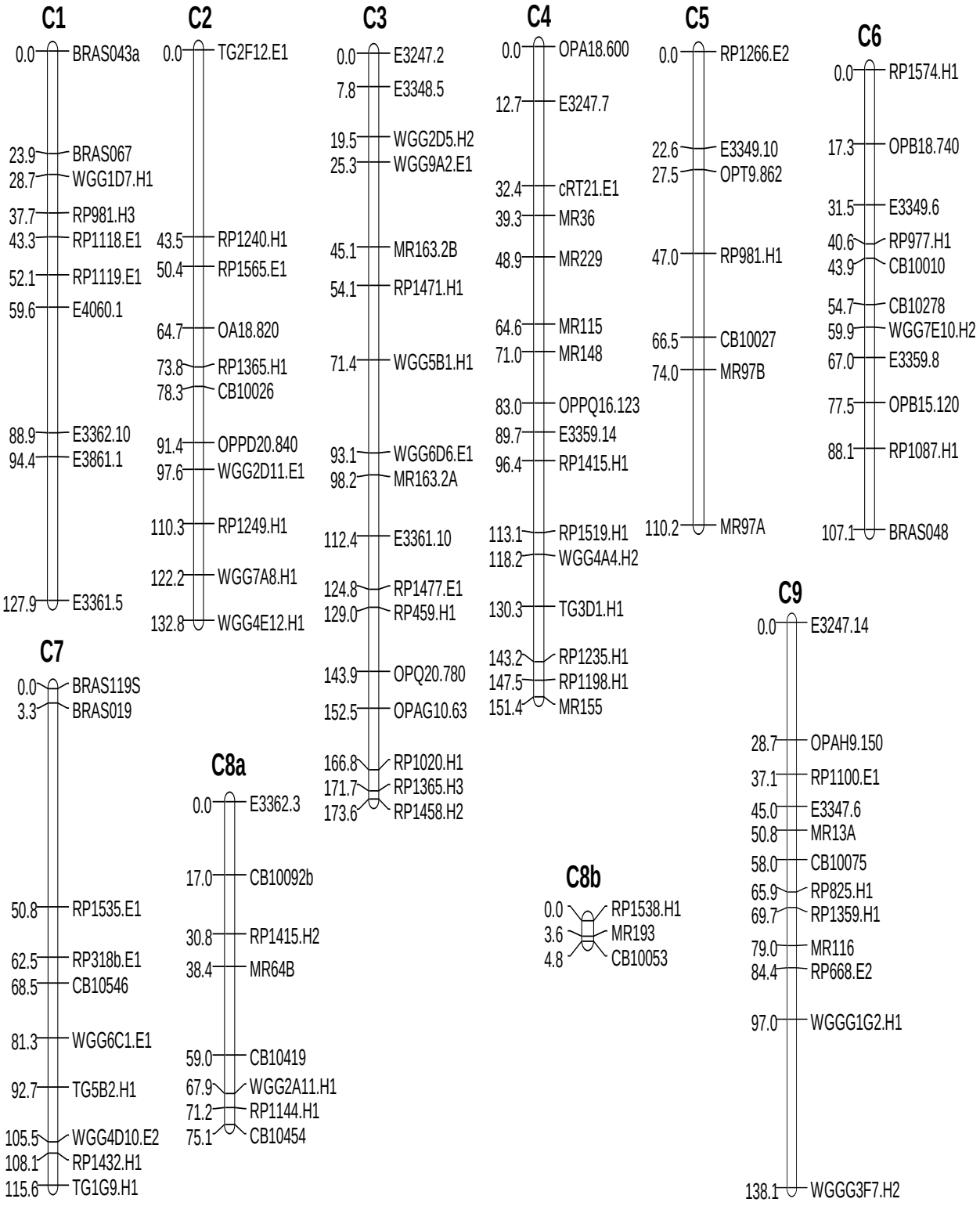


Figure III-5: Framework map of *Brassica napus* mapping population Mansholts × Samourai. The vertical bars represent linkage groups N1-N10 = A1-A10 and N11-N19 = C1-C9 (international nomenclature). Marker locus names and positions (cM) are located to the left and right of the vertical bars, respectively

Figure 5 continued from page 46



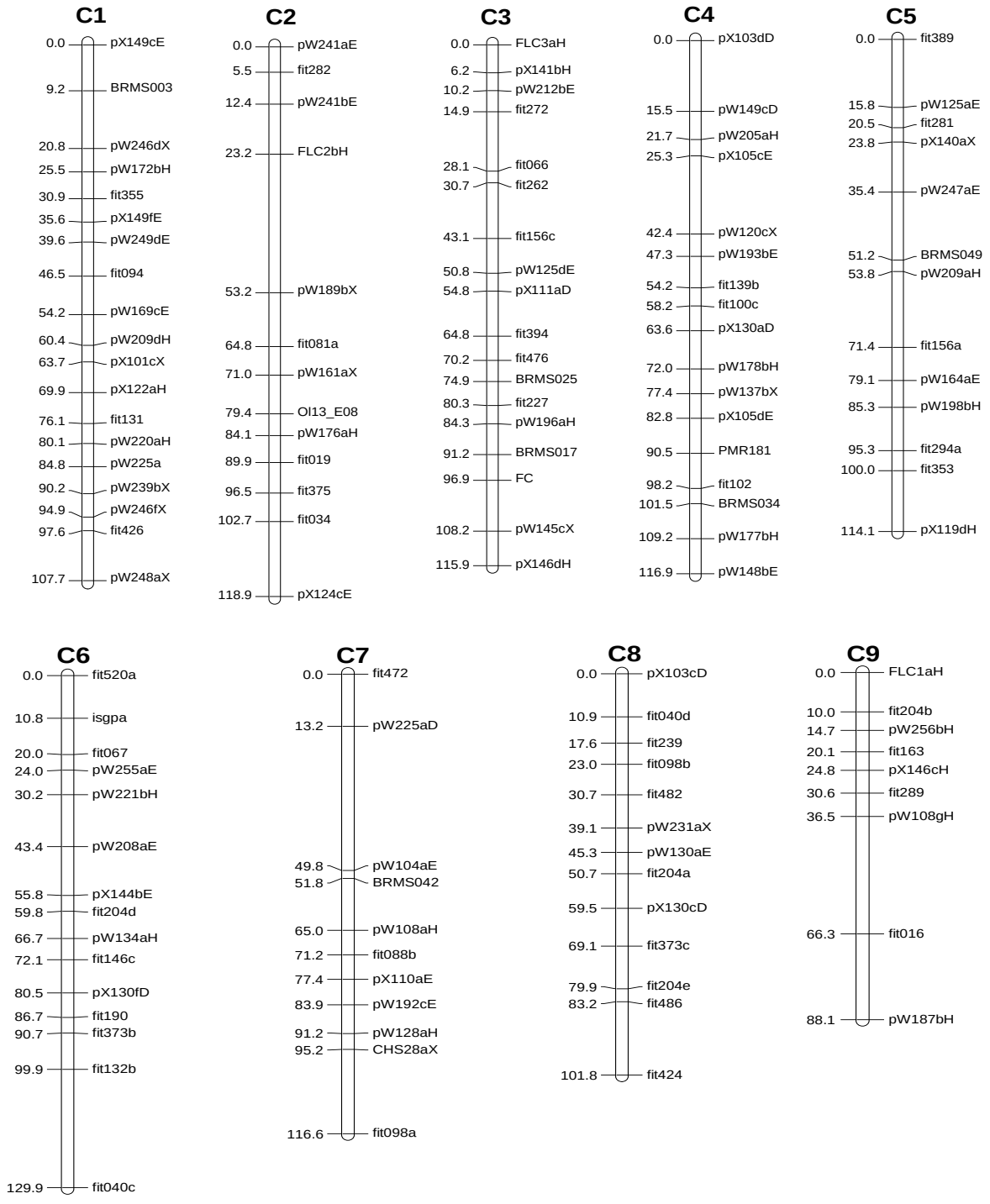


Figure III-6: Framework map of *Brassica oleracea* mapping population Bo1TBDH. The vertical bars represent linkage groups N11-N19 = C1-C9 (international nomenclature). Marker locus names and positions (cM) are located to the left and right of the vertical bars, respectively

3.4.3 QTL analysis and localization

For germination parameters under control and salt stress conditions, a total of 22 QTL were identified in the three tested populations. The full statistics data on the QTL detected in the three tested populations, additive effect, flanking markers, and the variation explained by each QTL and LOD score, are summarized in Tables 10, 11 and 12. The position of QTL on linkage groups (LG) is outlined in Figures 7, 8 and 9. Positive additive effect means that alleles of Alesi increase the threat while the negative additive effect means that the alleles of H30 increase the trait.

Brassica napus DH population (Alesi × H30)

Germination percentage

No QTL were identified under control conditions. Under salt stress one putative QTL, G%-1S, was detected on LG C1. This QTL explains 6% of the phenotypic variation with a negative additive effect.

Germination pace

Under control, three QTL, GP-1C, GP-2C and GP-3C were detected on LGs A9, C1 and C4, respectively. They explain 37% of the phenotypic variance. Two QTL- GP-1C and GP-2C, show a negative additive effect. QTL GP-2C was identified at P = 0.05 and alone explains 17% of the phenotypic variation. The third QTL, namely GP-3C, shows a positive additive effect. No QTL were identified for GP under salt stress.

Salt tolerance index

Three QTL were localized, i.e. G%STI-1 was found on LG A3 which explains 6% of the phenotypic variation with a negative additive effect. For GP, two QTL, GP-STI-1 and GP-STI-2, were detected on LGs A9 and A10, respectively. GP-STI-1S shows a positive additive effect and explains 7% of the phenotypic variation. In contrast, GP-STI-2 shows a negative additive effect and explains 8% of the phenotypic variation.

Brassica napus DH population (Mansholts × Samourai)

Germination percentage

Two QTL, G%-1C and G%-2C, were identified on LG C1 at 24 cM and 53 cM, respectively. Together they explain 17% of the genotypic variation. For both QTL G%-1C and G%-2C, the additive effect was negative. Under salt stress, one QTL was identified as G%-1S on LG C5, explaining 4% of the phenotypic variation with a negative additive effect.

Germination pace

One QTL (GP-1C) was detected under control conditions on LG A9. This QTL explains 6% of the phenotypic variation with a positive additive effect. Under salt stress, one QTL GP-1S was detected on LG A8, explaining 6% of the phenotypic variation with a negative additive effect.

Salt tolerance index

No QTL were mapped for G%-STI and only one QTL GP-STI-1 was identified on LG A8, which explains 5% of the phenotypic variation with a negative additive effect.

Comparing the traits variation of the two *B. napus* populations, the mean values and distribution of the traits is quite similar under control and salt stress. The number of QTL was quite similar, with seven QTL for the *B. napus* DH population (Alesi and H30), and six QTL for *B. napus* DH population (Mansholts and Samourai).

***Brassica oleracea* population Bo1TBDH**

Germination percentage

The QTL mapping resulted in three QTL: G%-1C, G%-2C and G%-3C. G%-1C was detected on LG C2, while G%-2C and G%-3C were found on the same LG C4 at 44 cM and 99 cM, respectively. The QTL values account for 30% of the phenotypic variation. Only G%-3C were mapped at P = 0.05. G%-1C and G%-2C show a positive additive effect. Under salt treatment, one QTL was identified for G%-1S on LG C1, which explains 7% of the phenotypic variation with a positive additive effect.

Germination pace

Under control, three QTL were mapped: GP-1C and GP-2C were found on LGs C4 at 40 cM and 102 cM, respectively. GP-3C was mapped on Lg C5. These three QTL together explain 30% of the phenotypic variation. The QTL GP-1C and GP-2C showed a positive additive effect. Conversely, the additive effect was negative for GP-3C. Under salt treatment, one QTL GP-1S was detected on LG C4, explaining 6% of the phenotypic variation with a positive additive effect.

Salt tolerance index

One QTL for G%: GP-STI-1 was detected on LG C3, which explains 8% of the phenotypic variation with negative additive effect.

Table III-10: QTL detected at LOD > 1.2 under control treatment (C) and Salt treatment (S) for germination percentage (G%), germination pace (GP), and respective salt tolerance indices (G%STI, GPSTI) in *Brassica napus* mapping population Alesi × H30. (QTL significant with *P* = 0.05 are marked bold)

Treatment	Trait	Name of QTL	LG	LOD	Position (cM)	interval	Flanking Markers	Additive Effect	Phenotypic Variation explained (%)
C	GP	GP-1C	A9	3.0	81	80-89	ra08600 -ra07944	-0.03	10.45
	GP	**GP-2C**	**C1**	**5.3**	**44**	**32-54**	**ra08390 -sN00983**	**-0.04**	**17.5**
	GP	GP-3C	C4b	2.6	11	0-22	MR155 -CB10335	0.03	8.79
S	G%	G%-1S	C1	1.8	54	43-79	sN00983-ra03282	-10.70	6.41
	G%-STI	G%-STI-1	A3	1.8	51	50-54	ra00527-sN08841	-10.56	6.31
	GP-STI	GP-STI-1	A9	2.0	81	80-89	ra08600-ra07944	4.54	7.27
	GP-STI	GP-STI-2	A10	2.2	56	35-57	CB10021-ra12416	-4.66	7.59

Table III-11: QTL detected at LOD > 1.2 under control treatment (C) and Salt treatment (S) for germination percentage (G%), germination pace (GP), and respective salt tolerance indices (G%STI, GPSTI) in *Brassica napus* mapping population Mansholts × Samourai. (QTL significant with *P* = 0.05 are marked bold)

Treatment	Trait	Name of QTL	LG	LOD	Position (cM)	interval	Flanking Markers	Additive Effect	Phenotypic Variation explained
C	G%	G%-1C	C1	1.8	24	8-27	BRAS067 - W1D7.H1	1.88	6.12
	G%	G%-2C	C1	3.4	53	52-58	RP1119.E1 -F4E4060.1	-2.48	11.28
	GP	**GP-1C**	**A9**	**1.8**	**14**	**13-16**	**MD41 - WG3F7.H1**	**0.02**	**6.06**
S	G%	G%-1S	C5	1.2	107	90-110	MR97B -MR97A	-5.99	4.31
	GP	GP-1S	A8	1.8	77	68-78	RP299.E1 -OPQ9.1590	-0.02	6.27
	GP-STI	GP-STI	A8	1.5	77	68-78	RP299.E1 -OPQ9.1590	-3.80	5.15

- The additive effect is calculated by subtracting Mansholts allele by Samourai allele.

Table III-12: QTL detected at LOD >1.2 under control treatment (C) and salt treatment (S) for germination percentage (G%), germination pace (GP), and respective salt tolerance indices (G%STI, GPSTI) in *Brassica oleracea* mapping population Bo1TBDH. (QTL significant with *P* = 0.05 are marked bold)

Treatment	Trait	Name of QTL	LG	LOD	Position (cM)	Interval	Flanking markers	Additive Effect	Phenotypic variation explained (%)
C	G%	G%-1C	C2	2.6	63	53-72	pW189bX -fit081a	-4.50	8.65
	G%	G%-2C	C4	1.6	44	32-48	pW120cX -pW193bE	3.70	5.34
	G%	**G%-3C**	**C4**	**4.1**	**99**	**92-102**	**fit102 -BRMS034**	**6.93**	**15.66**
	GP	GP-1C	C4	2.7	40	32-47	pX105cE -pW120cX	0.02	9.08
	GP	GP-2C	C4	3.3	102	98-108	BRMS034 -pW177bH	0.02	10.91
	GP	GP-3C	C5	3.1	114	109-114	fit353 -pX119dH	-0.02	10.45
S	G%	G%-1S	C1	2	90	84-95	pW225a -pW239bX	7.12	6.93
	GP	GP-1S	C4	1.8	91	83-99	PMR181 -fit102	0.01	6.07
	G%-STI	G%-STI	C3	2.0	28	21-31	Fit272- fit066	-13.02	8.44

- The additive effect is calculated by subtraction TO1000DH3 allele by Early Big allele.

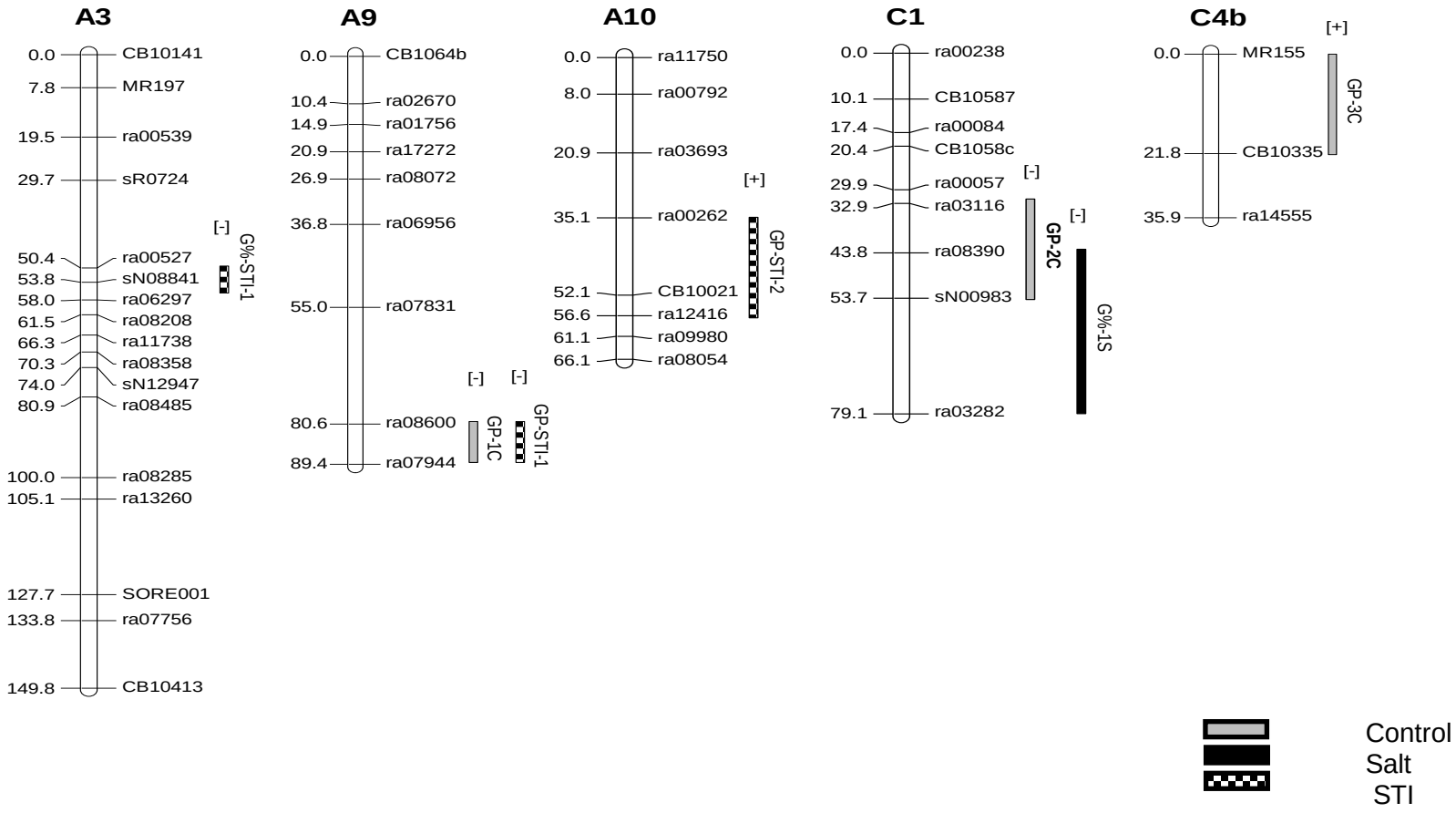


Figure III-7: Localization of QTL for germination parameters *in Brassica napus* DH population Alesi x H30. (QTL significant with *P* = 0.05 are marked bold)

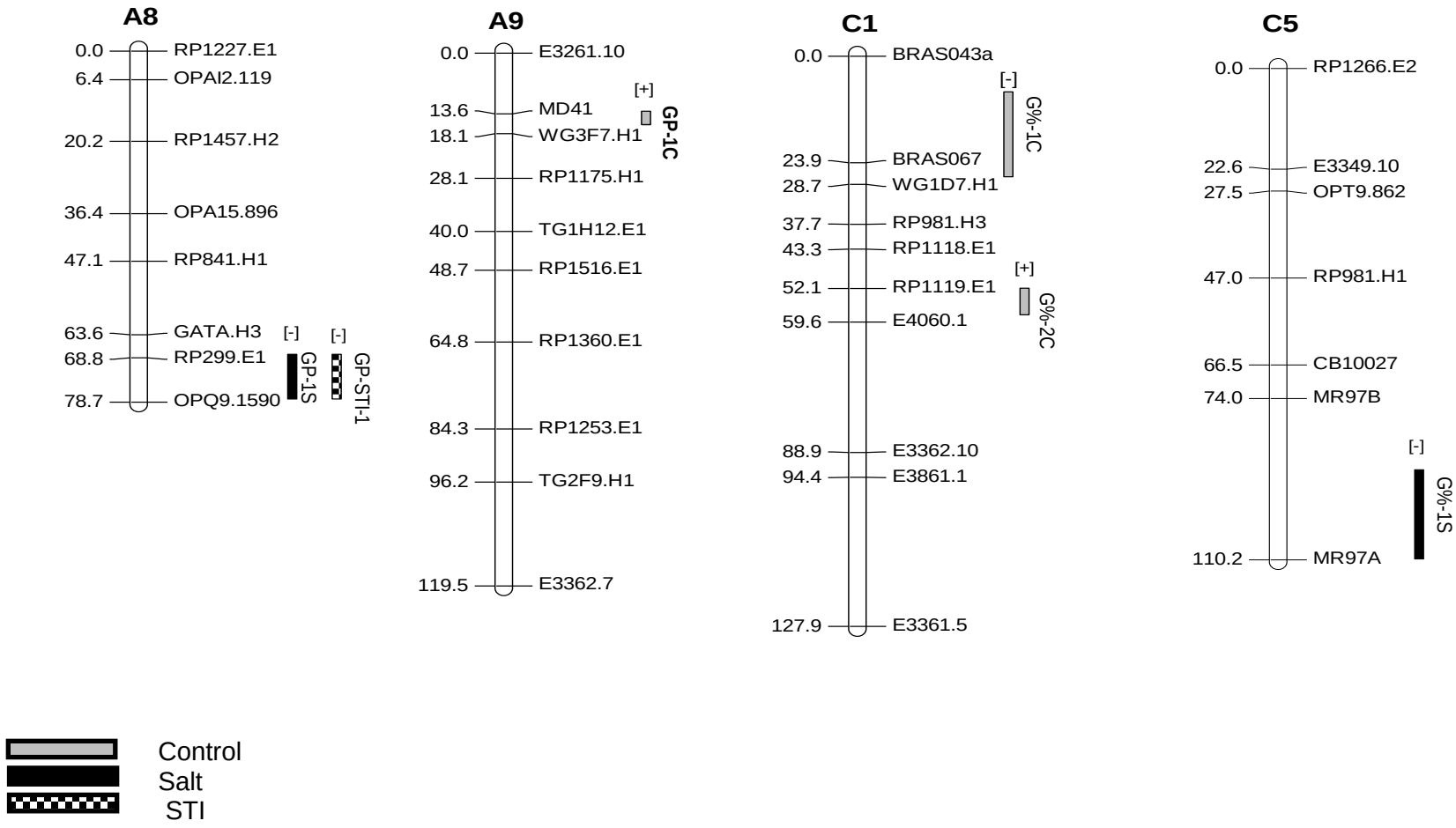


Figure III-8: Localization of QTL for germination parameters *in Brassica napus* DH population Mansholts x Samourai. (QTL significant with *P* = 0.05 are marked bold)

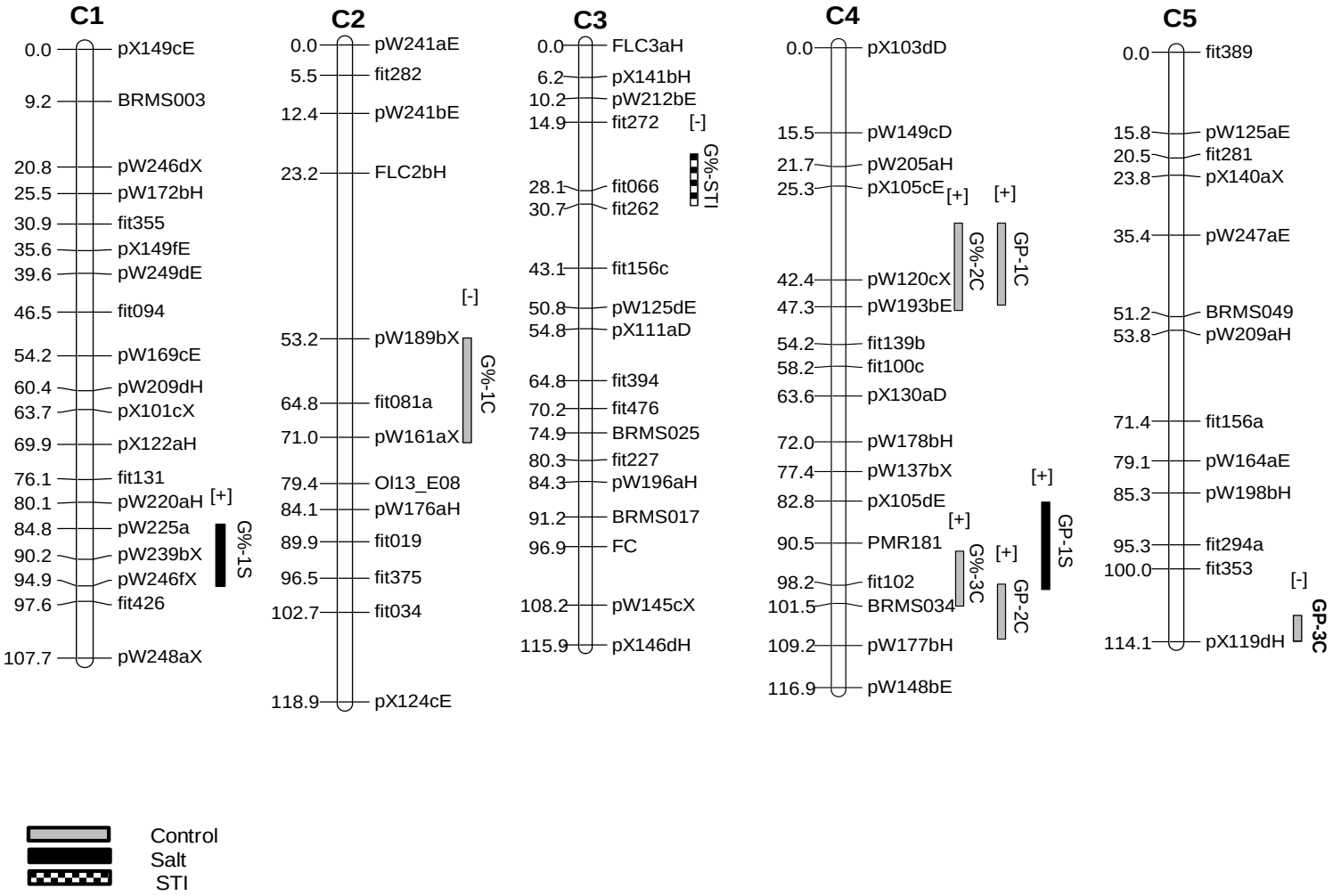


Figure III-9: Localization of QTL for germination parameters in *Brassica oleracea* Bo1TBDH population. (QTL significant with *P* = 0.05 are marked bold)

3.5 Discussion

3.5.1 Traits variations

In the present study, three traits were measured, namely germination percentage (G%), germination pace (GP), and salt tolerance index (STI). Our results indicate that the impact of salt stress was higher on GP than on G% because several DH lines germinated under salt stress with 100% germination. But the germination rate was slower in the salt stressed plants than in the controls. It is thus clear that no DH line showed the same GP under both treatments of control and salt. These findings were consistent with the results of Foolad and Jones (1991) in tomato. They found that salinity stress reduced the G% and retarded the germination rate, which increased the time to complete germination. The variations in the responses of populations to stress depend on the magnitude of the stress and the genetic background of each population (Foolad et al. 1999). Zheng et al. (1998) reported that seed germination and seedling establishment of *B. napus* is delayed and reduced under salinity. In tomato and *Arabidopsis*, similar results were reported, where salt stress reduced the germination rate, though several genotypes showed the maximum germination level (Foolad et al. 1999; Galpaz and Reymond 2010). A reduction and retardation of germination are mainly due to the high osmotic pressure surrounding the seeds. The high osmotic pressure surrounding the germinated seeds causes water deficiency. The accumulation of Na^+ and Cl^- induces nutrition deficiency, ion toxicity and catabolistic disturbance, or a combination of these; these hazards underlie the retardation of germination (Foolad 1999). Osmotic stress has more impact than ion toxicity. This assumption was strengthened by the findings of Huang and Redman (1995) report that polyethylene glycol (PEG) and NaCl equally affect equally the germination of *B. napus*. Similarly, in tomato, Foolad et al. (2003) proposed that osmotic pressure was more fatal than ion toxicity. They found a positive correlation between seed germination under both salt stress and drought stress. Furthermore, they found that the genotypes that were selected as salt tolerant also showed drought tolerance. These results suggest the presence of cross-talk between the mechanisms that regulate plant responses to the different abiotic stresses.

3.5.2 QTL analysis and localization

For each trait, one or more QTL were identified under control or stress conditions. For instance, in the population of Alesi and H30, two QTL, GP-1C and GP-STI-1, were co-localized on LG A9. Likewise, the two QTL, GP-2C and G%-1S, were co-localized on LG C1 (Figure 7). This co-localization is expected because these traits are related. Furthermore, STI expresses performance under control and salt stress. This co-localization of QTL on LG A9 indicates that in this genomic region there may be one gene with a pleiotropic effect or two tightly linked genes independently responsible for the variation on these traits. In both cases, on LG A9 and LG C1, the additive effect of the QTL was negative, meaning that the alleles on each LG are in a couple phase. One adaptive QTL GP-3C, which controls the variation under control conditions, was mapped on LG C4b. This means in this genomic region, gene(s) govern (s) the GP variation only under control conditions. Additionally, two constitutive QTL, G%-STI-1 and GP-STI-2 which regulate the variation of one trait under control and salt stress,

were mapped on LGs, A3 and A10, respectively. These results indicated that on LGs, A3 and A10, there are genomic regions that harbor gene(s) to control the GP variation under control and salt stress.

Similarly, in the Mansholts and Samourai population two QTL, GP-1S and GP-STI-1, were co-localized on LG A8 (Figure 8). Since, STI expresses the performance under control and salt stress, the QTL related to this trait were constitutive that regulate the variation of the corresponding trait under control and salt stress conditions. The overlapping of these QTL intervals suggests that one gene with pleiotropic effect lies behind the variation of this trait under control and salt stress conditions, or two closely linked genes that control the two traits independently. The additive effect of both QTL was negative. This means that these Mansholts alleles increase the corresponding traits and that they are in a couple phase. Additionally, four adaptive QTL were mapped on LGs; A9, C1 and C5, respectively.

No common QTL were mapped between the two populations of *B. napus,* which indicates that these QTL were population specific. The inconsistency in identifying QTL in different populations and in different environments can be attributed to a number of factors such as different sets of markers, genetic background of parental lines or population types (Collins et al. 2008). In the case of *B. napus* populations, the most likely explanation would be the difference in parental line sources: there was no common parental line between the two populations. This is consistent with the findings of Monforte et al. (1997) in tomato; they found that the QTL effect varies between populations, depending on the genetic background of the population. Moreover, the QTL effect changes in the presence/absence of salinity. This QTL explained 58% of fruit fresh weight under non-stress conditions. Under salt stress, this QTL explain 14% of the variation for the same trait.

In *B. oleracea*, the Bo1TBDH population the intervals of three constitutive QTL; G%-3C, GP-2C and GP-1S are overlapped on LG C4. Moreover, near the middle of this LG two adaptive QTL are co-localized, namely GP-1C and G%-2C (Figure 9). In these genomic regions there might be one gene with a pleiotropic effect controlling the variation in these traits. Another possibility is that three genes underlie these trait variations; the alleles for increasing the GP and G% are descended from TO1000DH3, which are in a couple phase because the additive effect of all QTL was positive. In *B. oleracea,* our results are in agreement with the results of Bettey et al. (2000), who detected four QTL under control condition on LGs: C1, C4, C5 and C6. They detected one QTL under stressful conditions, while we found two QTL; this discrepancy might be due to the different plant materials.

According to Collins et al. (2008), the QTL that were mapped in the three populations can be classified into two types; constitutive QTL, which exist and sustain their effect under both control and salt stress conditions, and adaptive QTL, which control the variation of one trait under either control or salt stress conditions. In all populations, both types were detected. These findings indicate that distinct genomic regions control

germination both under non-stress and stress conditions, while other genomic regions affect germination either under control or under stress. Our results are in agreement with the findings of other authors for different crops; in tomato (Foolad et al. 1999), in *Arabidopsis* (Quesada et al. 2002; Clerkx et al. 2004; Galpaz and Reymond 2010) and in *B. oleracea* (Bettey et al. 2000). These authors speculate that some genomic regions regulate the germination event under control and stress conditions; these QTL are termed stress-nonspecific or constitutive QTL. They also found salt-specific (adaptive) QTL.

A transgressive distribution was found for all traits (Figures 4, 5 and 6). This transgressive segregation indicates that the alleles responsible for increasing or decreasing a particular trait are scattered in the parental lines of each mapping population. Similar results concerning the contribution of salt-sensitive parents in increasing salt tolerance were found in *Arabidopsis* (Quesada et al. 2002, Clerkx et al. 2004, Galpaz and Reymond 2010), and in tomato (Foolad et al. 1999).

Conclusion

We observed a large variation in all investigated traits in the tested populations. The effect of salinity on seed germination may be attributed to osmotic stress or ion-toxicity or a combination of both. Mostly, the distribution of the traits was normal, with a transgressive segregation, meaning that both parents could contribute positively to increasing a particular trait. We mapped several QTL underlying seed germination traits such as germination percentage, germination pace and the performance of genotypes under control and salt stress. These results might prove helpful in understanding the genetic and physiological mechanisms that control salt tolerance in the seed germination stage of *Brassica* species. The markers associated with the mapped QTL can be employed for selecting the best genotypes without further phenotyping evaluation.

Of great importance is the presence of stress-nonspecific QTL that control the seed germination under control and salt stress conditions. Also, the fine mapping of these QTL might help us to uncover the causal genes that reside within their intervals and to understand their contributions.

Chapter IV

Mapping QTL for salt tolerance at the young plant stage and leaf glucosinolates in a *Brassica napus* DH population

4.1 Introduction

Brassica napus originated from interspecific hybridizations between turnip rape *(Brassica rapa* AA, 2n = 20) and cabbage (*Brassica oleracea*; CC, 2n = 18) that occurred spontaneously (Morinaga 1934; U 1935) during medieval times (Iñiguez-Luy and Federico 2011). It is thought to be a relatively new species, about 500 years old, and no wild populations have been recorded (Gómez-Campo and Prakash 1999). *Brassica napus* has been classified as a moderately salt tolerant plant (Mass and Hoffman 1977). Under salt stress, polyploid species of *Brassica* such as *B. napus* show superiority over diploid species (Mailk 1990; He and Carmer 1992; Ashraf et al. 2001). Several approaches have been pursued to enhance salinity tolerance in *Brassica,* such as conventional breeding, somaclonal variation and gene transfer (Purty et al. 2008). Until now, the QTL for salinity tolerance in *Brassica* species have never been reported, making it difficult to understand the genetic basis of salinity stress tolerance in *Brassica* species (Nayidu et al. 2013). A number of studies have reported successes in improving salt tolerance in *B. napus* by gene transfer (Huang et al. 2000; Prasad et al. 2000; Zhang et al. 2001; Srivastava et. al. 2004; Song et al. 2014). These reports demonstrate the considerable increase in salt tolerance that can be achieved by single gene overexpression, despite the fact that salt tolerance is a polygenic trait.

Brassica napus has a unique aliphatic glucosinolate profile (Mithen 2001). Our knowledge about the genetic control of leaf glucosinolates (GSL) variation in *B. napus* is rather limited compared to the genetic control of seed glucosinolates. The role of glucosinolates in biotic stresses such as insect attack and pathogens resistance has been energetically studied. In contrast, knowledge of the role of GSL under abiotic stressors such as light, drought, salinity and heat is still vague. Several environmental factors affect the concentration and composition of glucosinolates, such as light, drought, temperature and salinity (Qasim et al. 2003; Velasco et al. 2007; López-Berenguer et al. 2008; Mewis et al. 2012).

4.2 The objectives of this chapter are

1. To analyze the genetic variation of the *B. napus* DH population under salt stress and to map the QTL which regulate salt tolerance in *B. napus* in the young plant stage.
2. To study the variations in leaf glucosinolates under control and salt stress and to identify the QTL that underlie these variations.

Part I

Mapping QTL for salt tolerance at the young plant stage in *B. napus*

4.3 Materials and Methods

4.3.1 Plant material

The plant material consists of 138 doubled-haploid (DH) lines that were developed from crossing Mansholts Hamburger Raps (Mansholts) and Samourai. Mansholts has ++ quality, high erucic acid content and high glucosinolate content. Samourai has 00 quality, low erucic acid content and low glucosinolates content. A full description of this population is provided in Chapter 3 (3.3.1)

4.3.2 Greenhouse experiment and treats assessment

The 138 DH lines and their two parents were tested in the greenhouse in two consecutive experiments. The first replicate was sown on October 3, 2012 and harvested on November 5, 2012. The second replicate was sown on November 14, 2012 and harvested on December 19, 2012. The fluctuation in temperature for each replicate and the mean overall temperature and time courses of the two replicates are shown in Appendix 7.

To guarantee homogenous germination, four seeds were sown in 7x7 cm pots filled with 50% soil 50% sand mixture. Each genotype was represented by 5 adjacent pots. The temperature was automatically adjusted to 20°C during the day and 15°C at night. The relative humidity was approximately 47%. Plants were exposed to long day conditions, i.e. 16 h light and 8 h dark. In addition to daylight, each table was illuminated by two 400-watt SON-T-Agro sodium vapor lamps (Phillips, Netherlands). The average light intensities were 244 µmol/m2*s for experiment one and 203 µmol/m2*s for experiment two. The light intensity was measured with Sunscan SS1 (Delta-T, England). The pots were placed on ten tables in the greenhouse. Seven genotypes, namely the two parents and five DH lines were placed randomly on each table and used for checking. A single row of pots with the commercial cultivar Elektra was placed on along the edges of each table to avoid border effects.

4.3.3 Germination and establishment

After sowing, the pots were watered with 100 ml tap water per pot. To keep the soils thoroughly moist and to avoid poor aeration conditions, they were watered by overhead spraying from days seven to day 12. The first expanded leaf emerged 13 days after sowing (das). The seedlings were thinned into two seedlings per pot. On the 15^{th} das, the watering method was changed to the flooding method where plants received water from the underneath. Each table was filled with 60 l tap water; the water height was about 2 cm for 2 hours. The water was supplemented with 0.5 g/l of the compound fertilizer Hakaphos blue (COMPO, Netherlands).

4.3.4 Salt stress

For the salinity treatment, the plants were placed on five tables and watered 23 das, once with a saline solution of 100 mM NaCl, for acclimation. Each table was filled with

60 l saline solution to a height of about 2 cm. The saline solution was enriched with 1 g/l of Hakaphos blue. At day 27, the concentration of the saline solution was increased to the final concentration of 200 mM NaCl enriched with 1 g/l of Hakaphos blue and the plants were watered by flooding as described above twice a week until the end of the experiment at 35 das. Beginning on the 23rd das, the plants on the five tables serving as a control group were watered with the same amount of tap water enriched with 1 g/l of Hakaphos blue until the end of the experiment.

4.3.5 Harvest and biomass

Thirty-five das, the above-ground parts from four pots were harvested and bulked, and the fresh weight was recorded. All genotypes of each replicate were harvested on the same day. Dry weight was recorded after samples were dried an oven at 60°C for 72 hours.

4.3.6 Relative water content

The relative water content was calculated as

$$\mathrm{RWC} = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{FW}} \text{ x } 100$$

4.3.7 Chlorophyll content

The chlorophyll content was measured twice with a SPAD-Meter Minolta 502 (Osaka, Japan). The first time was six days after start of salt stress (SPAD1) and the second was 12 days after start of salt stress (SPAD2).

4.3.8 Sodium (Na^+) and Potassium (K^+) analysis

For the sodium and potassium concentration measurements, the dried samples were pulverized using a coffee grinder (KRUPS 75, Germany). The dried samples of each genotype from replicate one and replicate two were combined. One day before digestion the samples were dried overnight at 100°C. From each sample 300 mg were placed in the cups of the MLS-MEGA II microwave system (Leutkirch, Germany). Four ml 65% HNO_3 and 2 ml H_2O_2 30% (Roth, Germany) were added to each sample. The samples were placed in the microwave system at 200°C for 55 minutes under 15 atm (atmospheric pressure) and cooled for 20 minutes. After digestion, the samples were diluted up to 25 ml using deionized and filtered Seralpur water. Samples were further diluted 1:10 (0.5 ml plant material extract + 4.5 ml Seralpur water). Na^+ and K^+ concentrations were measured using the flame photometer Eppendorf, Elex 6361 (Hamburg, Germany). The flame photometer was calibrated every ten samples using two calibration standards for both elements; low standard (0 mg/ l Na^+ and 0 mg/ l K^+) and high standard (100 mg/ l Na^+ and 100 mg/ l K^+). The Na^+ and K^+ content were calculated as mg g^{-1} DW, and then the Na^+/ K^+ ratio was calculated.

4.3.9 Glucosinolates analysis

At 34 das, one pot was harvested for leaf glucosinolates analysis. The leaves were frozen immediately in liquid nitrogen and stored at -20°C until analysis. Leaf

glucosinolate profile was measured by HPLC (High Pressure Liquid Chromatography) following the method of Kräling et al. (1990). The accuracy of the measurements was controlled by analyzing a seed sample from the standard cultivar Linetta once along with every 20 samples. Samples were lyophilized for 96 h in the freeze dryer Epsilon 2-40, Christ (Osterode, Germany). The samples were pulverized in a shaker with 4 2.3-mm balls; 200 mg of the pulverized plant material were weighed in a 13 ml polypropylene tube. For the extraction step, three ml of 70% methanol and 200 µl of internal standard (6 mmol L^{-1}) glucotropeolin were added. The tubes were vortexed and incubated (10 min, 75°C) in a water bath. The samples were shaken occasionally. After extraction, samples were centrifuged (5 minutes; 4000 rpm (rounds per minute)). The supernatants were transferred into empty labelled tubes. For the second extraction, two ml of 10% methanol were added to the pellets, and then the tubes were vortexed and incubated (10 min, 75°C) in a water bath. The samples were shaken occasionally. After centrifugation (5 minutes, 4000 rpm), each supernatant was combined and mixed with the preceding one, and the pellets were discarded. After that, 500 µL of the extract were pipetted on a column filled with 20 mg DEAE Sephadex A-25(S 9626; Sigma-Aldrich, D-82024 Taufkirchen). The column was washed twice with one ml deionized water. For desuphatising, 100 µL of sulphatase (Sigma- Aldrich) solution were added, and then the columns were incubated overnight at 39°C. The columns were flushed 3 times with 500 µL deionized water to elute the desulpho-glucosinolates in three ml polypropylene tube. 700 µL were pipetted in the HPLC vials for HPLC (125 9 3 mm Nucleodur 100-3 C18ec column; Machery-Nagel GmbH & Co KG, D-52313 Düren) analysis followed by UV detection (UV–VIS Detector L4250 Merck Hitachi).

4.3.10 Statistics

Before the analysis of variance, the value of each replicate's genotype, in the growth traits experiments as well as in the glucosinolate analyses, was adjusted using the moving average method implemented with the software PlabSTAT 3.0 (Utz, 2003). The model is described in Chapter 3 (3.5).

Trait abbreviations

Fresh weight (g) FW
Dry weight (g) DW
Relative water content RWC
Chlorophyll content measured by SPAD1 and SPAD2
Sodium content (mg/ g DM) Na^+
Potassium content (mg/ g DM) K^+
Sodium /Potassium ratio Na^+/K^+
Dry matter DM

Parental lines abbreviations

M= Mansholts S = Samourai

4.4 Results I

4.4.1 Traits variations

Minor differences were observed between the parental lines with regard to all of the traits measured: FW, DW, RWC, SPAD1, SPAD2, Na^+ mg/ g DM, K^+ mg/ g DM and Na^+/K^+ under both control and salt stress conditions, but these variations were notably wide in the DH population. Additionally, the variation in DH lines for the FW under salt stress was greater than that under the control. Under both conditions, a large number of DH lines showed lower biomass yield than the parental lines. Nevertheless, there was a transgressive segregation (Figure 1a, b, c, d, e, f). The genetic variance and the heritability estimated from the analysis of variance (ANOVA) for each trait under control and stress conditions are summarized in Table 1 and the maximum, minimum and mean values in Appendix 6.

Fresh weight

Under control conditions, out of 138 DH lines, 11 DH lines (7.9%) had a higher biomass yield than the parent with the high biomass yield, namely Mansholts. A large number 117 (84%) DH lines had lower values than the parent with the low biomass yield, i.e. Samourai. The minimum was 2.1 g and the maximum was 7.32 g. Under salt stress, 26 DH lines (18%) produced higher FW than the high-FW-yielding parent, Mansholts, and 88 DH lines (63%) had FW values lower than the low-biomass-yielding parent, Samourai (Figure 1a, b).

Dry weight

Under control conditions, 126 DH lines (91%) showed a DW lower than the weaker parent, Samourai, whereas only 10 DH lines (7%) revealed a DW higher than the stronger parent, Mansholts. The DW values ranged from 0.34 g to 0.93 g. Even with salt stress, the performance of seven DH lines (5%) was better than that of the high-performance parent, Mansholts. 129 DH lines (94%) exhibited weak performance, giving a DW lower than that of the parent with low dry weight, i.e. Samourai (Figure 1c, d).

Relative water content

The distribution of RWC shows the presence of transgressive segregation (Figure 2a, b). Under control conditions, 42 DH lines (30%) had a higher RWC value than the parent with the high value, and 69 DH lines (50%) had lower values than the parent with the lower RWC. The values ranged from 82 to 92. Under salt stress, only one genotype had a lower value than Samourai, whereas, a large number (122 = 88%) of the DH lines had higher values than the tolerant parent, Mansholts.

Chlorophyll content measured by SPAD

Figure 3a, b, shows the distribution of chlorophyll content measured under control and salt stress conditions for SPAD1 six days after starting the salt stress. Under control conditions, 125 DH lines (92%) had lower values than Samourai and 3 DH lines (2%) showed values higher than Mansholts; the range was from 20 to 44. Under salt stress, 113 DH lines (81.8%) showed values lower than Samourai and 8 DH lines (6%)

exhibited values higher than Mansholts; the values varied from 36 to 49. SAPD2 values were measured 12 days after applying salt stress. Regarding the controls, 79 DH lines (57%) had SPAD values lower than Samourai and eight DH lines (6%) showed values greater than Mansholts. The minimum and maximum values were 20 and 45, respectively. For salinity, the distribution was similar to that of the controls; with 79 DH lines (57%) having SPAD values lower than Samourai and eight DH lines (6%) showing values greater than Mansholts. An increase in SPAD values under salt stress was observed, the SPAD2 values ranging from 36 to 53 (Figure 3c, d).

Sodium content

Figure 4a, b shows the distribution of Na^+ mg/ g DM. A transgressive segregation was observed under control and salt stress. Regarding control, 48 (35%) of the DH lines were lower than the parent with low Na^+ content (Mansholts) and 64 (46%) of the DH lines had Na^+ content higher than the parent with high Na^+ content (Samourai). The values ranged from 0.76 mg/ g DM to 2.4 mg/ g DM. Under salt stress, a dramatic increase in Na^+ was observed compared to the Na^+ content under control. Out of 138 DH lines, 56 DH lines (41%) had Na^+ content lower than Mansholts, and 70 (51%) of the DH lines had a Na^+ content higher than Samourai. The range here was between 14.6 mg/ g DM and 36 mg/ g DM.

Potassium content

Compared to the difference in sodium content between controls and salt stressed plants, the difference between the potassium levels of plants under control and salt stress conditions was small. For the controls, it varied from 34 mg/ g DM to 81 mg/ g DM and for plants under salt stress it ranged from 30 mg/ g DM to 63 mg/ g DM. The distribution of K^+ content in the DH lines showed a transgressive segregation under control conditions, where 53 DH lines (38%) of the population showed values lower than Mansholts, the parent with the lower K^+ content. A set of 62 DH lines (45%) exhibited higher values than Samourai, the parent with the high K^+ content. Under salt stress, 65 DH lines (47%) exhibited a K^+ content lower than that of the parent with low K^+ content (Mansholts) and 64 DH lines (46%) exceeded the value of the parent with high K^+ content (Samourai) (Figure 4c, d).

Sodium Potassium ratio

Under control conditions, 42 DH lines (30 %) exhibited Na^+/K^+ lower than Mansholts, the parent with low Na^+/K^+, whereas 77 DH lines (55%) had a higher Na^+/K^+ than Samourai, the parent with high Na^+/K^+. Under salt stress, a group of 62 DH lines (45%) had lower values than Mansholts and 43 DH lines, accounting for 31%, showed Na^+/K^+ values higher than Samourai (Figure 4e, f). Regarding the control, the differences between genotypes ranged from 0.02 to 0.07. The variations between the DH lines under salt stress ranged between 0.27 and 0.76.

4.4.2 Correlations

The developmentally related traits revealed positive and significant correlations. For example, the correlation between FW and DW was high and significant under both

conditions (r = 0.75**) and (r = 0.86**) for control and salt conditions, respectively. Likewise, SPAD1 and SPAD2 were positively and significantly correlated (r = 0.67**), and (r = 0.72**) under control and salt conditions, respectively (Tables 2 and 3). The correlation between FW and other traits was positive but non-significant with SPAD1, SPAD2, K^+ and Na^+/K^+ under control conditions (Table 2 and 3). Under salt stress, the correlation of FW was negative and significant (r = -0.21*) and (r = 0.20*), for Na^+ and Na^+/K^+ respectively. Under control conditions, DW correlated positively and significantly (r = 0.20*) and (r = 0.20*) for SPAD1 and SPAD2 respectively. Under salt stress, the correlation for DW was positive and significant (r = 0.33*) and (r = 0.24*) for SPAD1 and SPAD2, respectively. The remaining traits showed a negative significant correlation with DW, with the exception of K^+ , which showed a positive significant correlation under salt stress (Tables 2, and 3). The correlation between RWC and all traits was negative and significant, except for Na^+, where it was positive (r = 0.44**) and (r = 0.34**) under control and salt stress, respectively. The RWC showed a positive and significant correlation with K^+ (r = 0.69**) and (r = 0.23**) under control and salt stress, respectively (Tables 2 and 3). The correlation of SPAD1 and SPAD2 under control and under salt stress was significant and negative with the remaining traits. Under salt stress, the correlation of SPAD1 and SPAD2 was negative and significant only with RWC (Tables 2 and 3). Under control conditions, the correlation of Na^+ content was weak and positive with FW and DW, whereas the correlation of SPAD1 and SPAD2 was negative and significant. Under salt stress, the correlation between Na^+ content for the remaining traits was negative and significant, but for RWC, the correlation was positive and significant (Tables 2 and 3). Under control conditions, K^+ correlated positively and significantly with DW, RWC, and Na^+ content, but negatively and significantly with SPAD1 and SAPD2. Under salt stress, the correlation between K^+ and the remaining traits was positive, except for SPAD1 and Na^+ (Tables 2 and 3).

Table IV-1: Mean squares and F test of significance from the ANOVA and heritabilities of fresh weight (FW), dry weight (DW), relative water content, chlorophyll content measured by SPAD (SPAD1, SPAD2) of *Brassica napus* DH population Mansholts x Samourai, 138 DH lines were tested under control conditions and at salt treatment (200 mM NaCl)

	Control				Salt			
Sources of Variance	Genotypes (G)	Replicates (R)	G ×R	h^2	Genotypes (G)	Replicates (R)	G ×R	h^2
DF	137	1	137		137	1	137	
FW(g)	0.56	44.58**	0.45	1.41	0.16	39.62	0.14	11.10
DW(g)	0.02**	5.81**	0.01	39.06	0.01*	1.67**	0.03	32.67
RWC	3.05**	1118.33**	1.11	63.39	1.55**	160.29	0.39	74.67
SPAD1	13.04**	1091.62**	4.90	62.23	12.67**	2224.87**	6.56	48.17
SPAD2	10.98**	156.62**	4.67	58.02	18.48**	10.87	6.07	67.11
Na+ mg/ g DM	0.44	-	-	-	20.34	-	-	-
K+ mg/ g DM	56.65	-	-	-	22.89	-	-	-
Na+/ K+	0.001	-	-	-	0.001	-	-	-

** Significant at *P* =0.01; * significant at *P* =0.05 and + significant at *P* =0.1

- For Sodium content (Na^+ mg/ g DM), Potassium content (K^+ mg/ g DM) and Sodium /Potassium ratio (Na^+/ K^+) the dry matter of each genotype of replicate one was combined with replicate two, therefore there were no replication and no heritability was calculated for these traits.

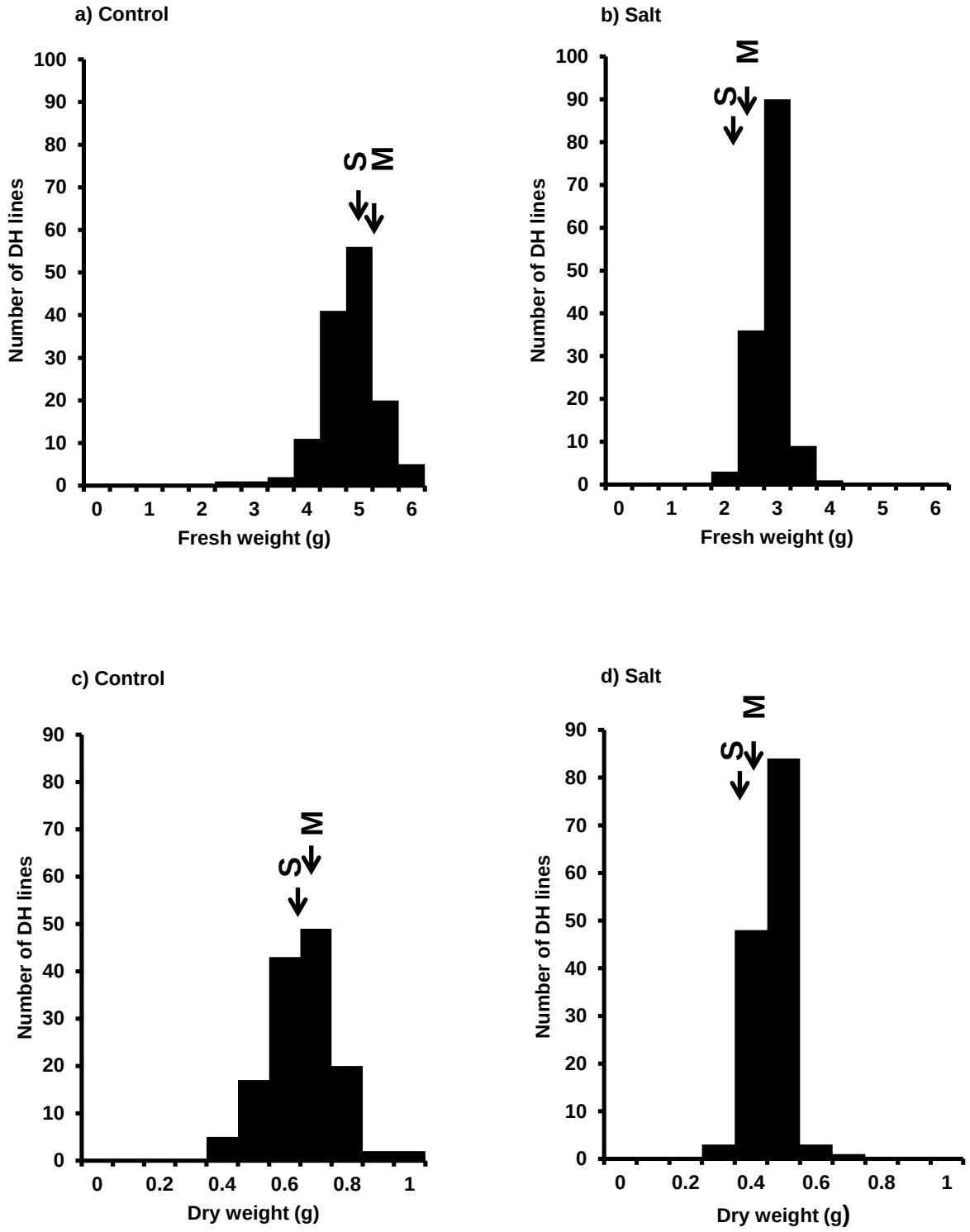


Figure IV-1: Frequency distribution of fresh and dry weight of *Brassica napus* DH population Mansholts × Samourai. a) Fresh weight under control, b) Fresh weight under salt stress, c) Dry weight under control and d) Dry weight under salt stress

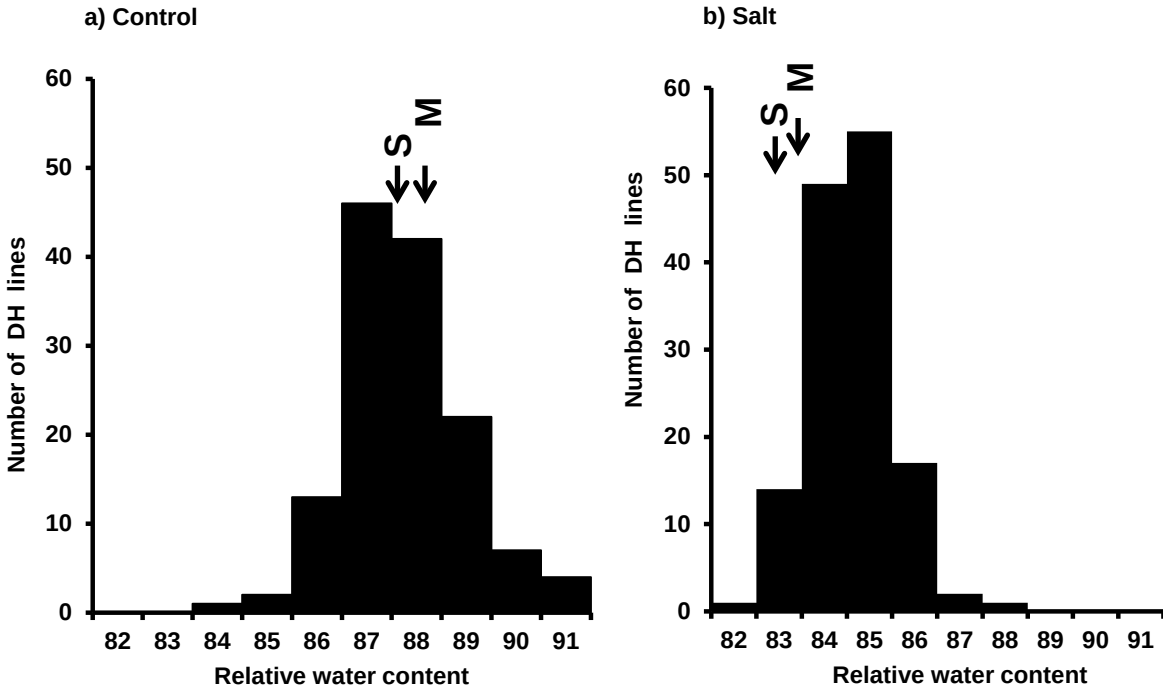


Figure IV-2: Frequency distribution of relative water content of *Brassica napus* DH population Mansholts × Samourai. a) Relative water content under control and b) Relative water content under salt stress

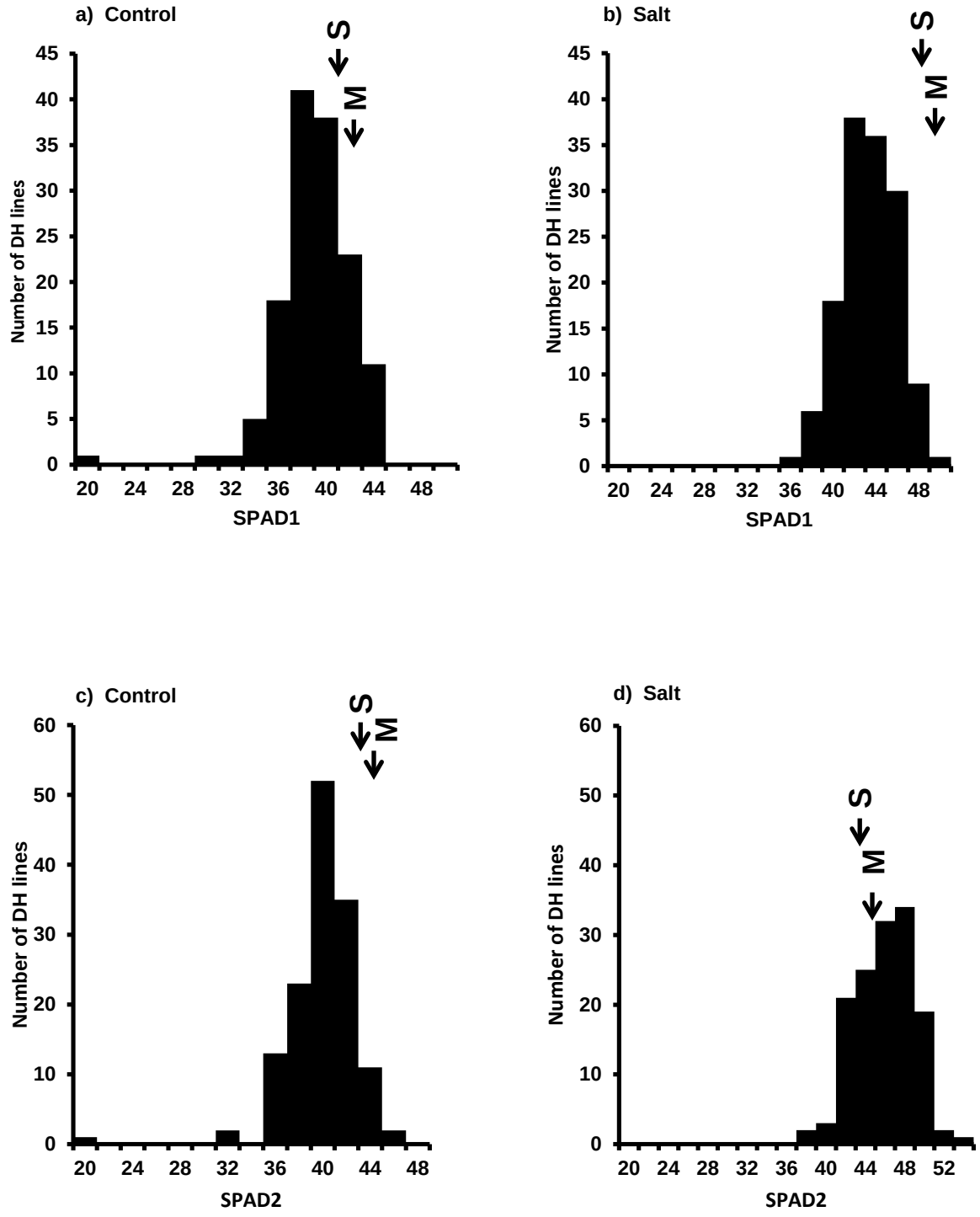


Figure IV-3: Frequency distribution of chlorophyll content measured by SPAD of *Brassica napus* DH population Mansholts × Samourai. a) SPAD1 under control and b) SPAD1 under salt stress, c) SPAD2 under control and d) SPAD2 under salt stress

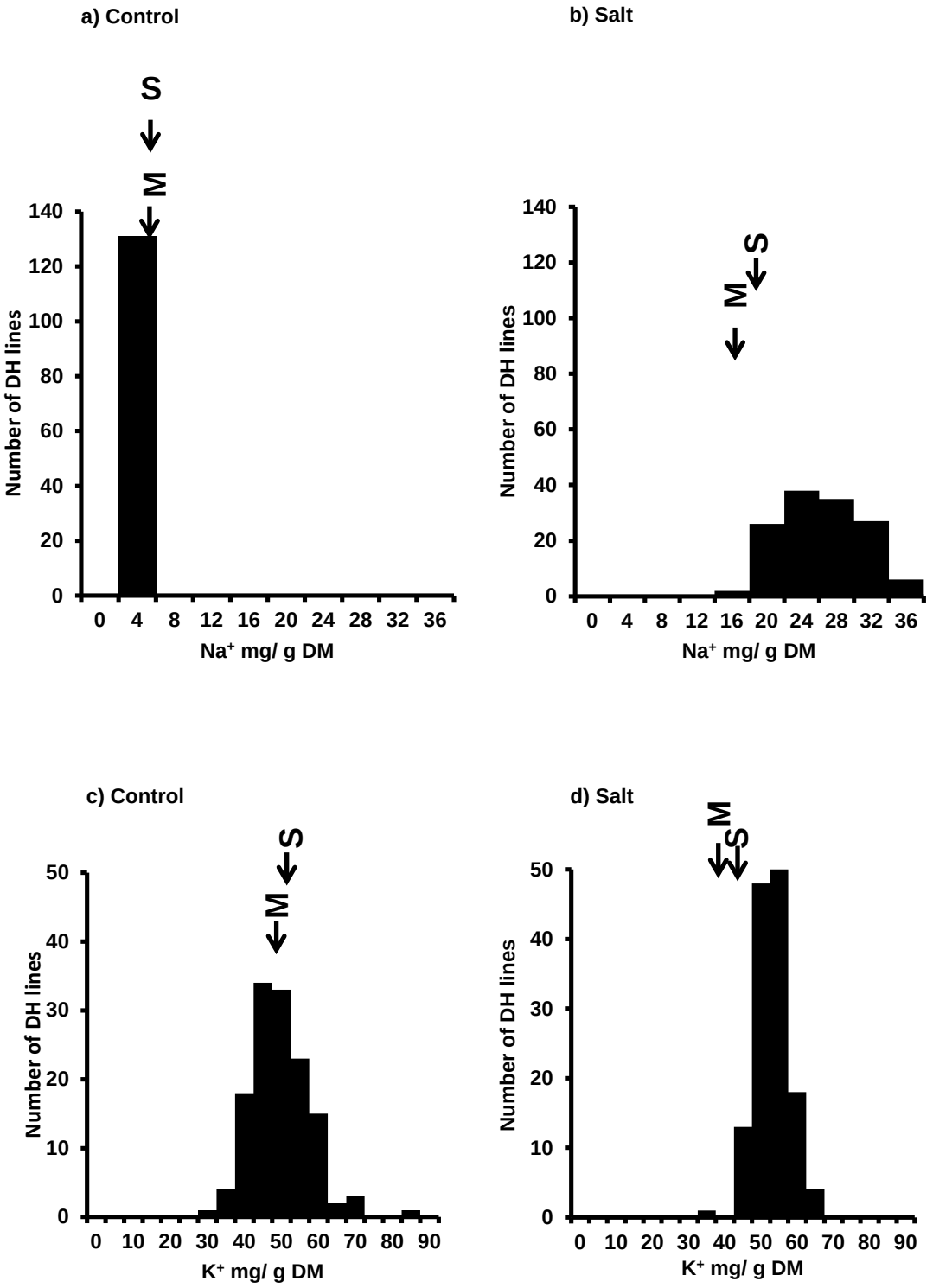


Figure IV-4: Frequency distribution of sodium content Na^+ mg/ g DM and potassium content K^+ mg/ g DM of *Brassica napus* DH population Mansholts × Samourai. a) sodium content at control and b) Sodium content under salt stress, C) potassium content under control and d) Potassium content under salt stress, e) Na/K ratio under control and f) Na/K ratio under salt stress

Figure IV-4 continued from page 70

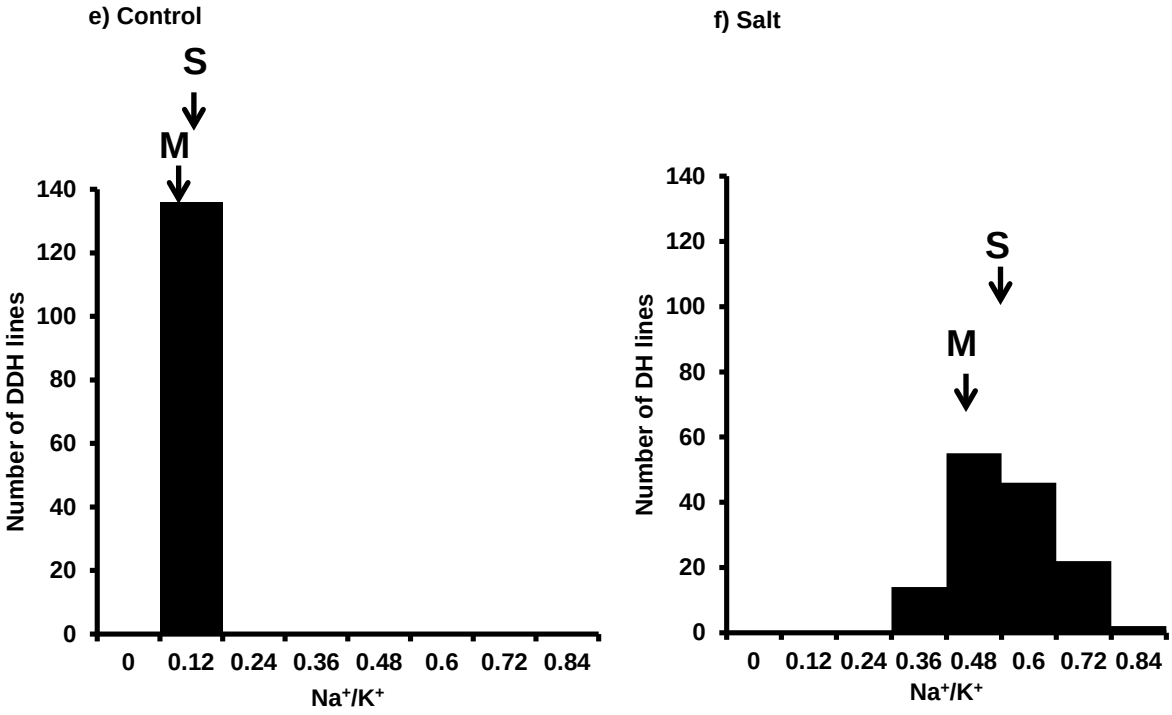


Table IV-2: Spearman's rank correlations of the estimated traits in *Brassica napus* mapping population Mansholts × Samourai under control

	FW(g)	Dw (g)	SPAD1	SPAD2	RWC	Na^+ mg/ g DM	K^+ mg/ g DM
Dw (g)	0.75**						
SPAD1	0.03	0.20*					
SPAD2	0.10	0.2*	0.69**				
RWC	-0.10	-0.61**	-0.30**	-0.29**			
Na^+ mg/ g DM	-0.10	0.30	-0.29**	-0.18*	0.43**		
K^+ mg/ g DM	0.13	0.58**	-0.26**	-0.20*	0.68**	0.67**	
Na^+ /K^+	0.001	0.06	-0.20*	-0.10	-0.03	0.60**	-0.10

Table IV-3: Spearman's rank correlations of the estimated traits in *Brassica napus* mapping population Mansholts × Samourai under salt stress (200 mM NaCl)

	FW(g)	Dw (g)	SPAD1	SPAD2	RWC	Na^+ mg/ g DM	K^+ mg/ g DM
Dw	0.86**						
SPAD1	0.14	0.32**					
SPAD2	0.08	0.23**	0.72**				
RWC	-0.05	-0.47**	-0.38**	-0.30**			
Na^+ mg / g DM	-0.20*	-0.34**	-0.04	-0.12	0.33**		
K^+ mg / g DM	0.06	0.17*	-0.08	0.03	0.28**	-0.11	
Na^+ /K^+	-0.20*	-0.03	-0.01	-0.11	0.18*	0.90**	-0.50**

4.4.3 QTL analysis and localization

The framework maps and the QTL mapping protocol are described earlier in Chapter 3 (3.3.4).

Twenty-eight QTL were identified for FW, DW, RWC, SPAD1, SPAD2, Na^+ mg/ g DM, K^+ mg/ g DM and Na^+ /K^+. Under control conditions, 13 QTL were identified for all traits and 15 for all traits under salt stress. The localization, additive effect, names of the mapped QTL on (LGs) for all traits under control and salt stress are shown in Figure 5. More details on the flanking markers, intervals, additive effect and logarithm of odds (LODs) are summarized in Tables 4 and 5. Where the additive effect is positive, this means that the Mansholts alleles increase the corresponding trait, while a negative additive effect indicates that the Samourai alleles increase the trait.

Fresh weight

Two QTL were identified. One is the QTL under control conditions FW-1C on LG A6, which explains 5% of the phenotypic variation with a negative additive effect. The second QTL was scored under salt stress on LG C3, which explains 7% of the phenotypic variation with a positive additive effect.

Dry weight

Five QTL were detected; three of which were under control conditions: DW-1C, DW-2C and DW-3C on the LGs A5, C2 and C3, respectively, accounting for 31% of the phenotypic variation. DW-2C was mapped at a significance level of P = 0.05 and explains 17% of the phenotypic variation. The additive effect was negative for DW-1C and positive for DW-2C and DW-3C. Under salt stress, two QTL, DW-1S and DW-2S, were localized on the LGs C3 and C6, respectively. They jointly explained 18% of the phenotypic variation. The two QTL were detected at significance level P = 0.05 with a positive additive effect.

Relative water content

Under control conditions, two QTL, RWC-1C and RWC-2C, were identified on the LGs C2 and C3, respectively. Together, they explain 26% of the phenotypic variation with a negative additive effect for both of them. RWC-1C was recognized at a significance level P = 0.05 and alone accounted for 17% of the explained phenotypic variation. Under salt stress, one QTL, namely RWC-1S, was found on LG C3, which explains 8% of the phenotypic variation with a negative additive effect.

Chlorophyll content after six days of salt stress (SPAD1)

QTL mapping for this trait identified six QTL. For the controls, three QTL, SPAD1-1C, SPAD1-2C and SPAD1-3C, were detected on the LGs A9, C2 and C6, respectively. The additive effects of all QTL were positive. These QTL jointly explain 43% of the phenotypic variation. SPAD1-1C, SPAD1-2C were mapped at P = 0.05. Likewise, under salt stress, three QTL, SPAD1-1S, SPAD1-2S and SPAD1-3S, were mapped on the LGs A1, A9 and C2, respectively. SPAD1-3S was localized at P = 0.05 and explains 15% of the phenotypic variation. Collectively, these three QTL explain 37% of

the phenotypic variation. SPAD1-2S and SPAD1-3S show a positive additive effect, while SPAD1-1S shows a negative additive effect.

Chlorophyll content after 12 days of salt stress (SPAD2)

The highest number of QTL was mapped for the chlorophyll content trait. Nine QTL were mapped, of which two QTL were for the controls and the remaining seven QTL for salt stress. For the controls, SPAD2-1C and SPAD2-2C were localized on the LGs A9 and C2, respectively. They explain 21% of the phenotypic variation, where SPAD2-2C was mapped at P = 0.05 and accounts for 14% of the phenotypic variation. Both QTL had positive additive effects. Under salt stress, seven QTL were localized, namely SPAD2-1S, SPAD2-2S, SPAD2-3S, SPAD2-4S, SPAD2-5S, SPAD2-6S and SPAD2-7S. They collectively explain 93% of the phenotypic variation. Two QTL, SPAD2-4S and SPAD2-5S, were mapped at P = 0.05 and disclose 47% of the phenotypic variation. These two QTL were identified at the LGs A9 and C2, respectively. Both of them had positive additive effects. The other five were mapped on the LGs A1, A3, A7, C3 and C3, respectively. They account for 46% of the phenotypic variation. The additive effects for the five QTL were negative.

Sodium content

No QTL were identified for under control conditions. Under salt stress, two QTL, Na-1S and Na-2S, were mapped on the LGs A3 and C9. They explained 21% of the phenotypic variation, with negative additive effects.

Potassium content

Under control conditions, the two QTL, K-1C and K-2C, were mapped on the LGs C2 and C3, respectively. The QTL K-1C was mapped at P = 0.05, which explains 18% of the phenotypic variation, while K-2C explains 7% of the phenotypic variation. The additive effects for both QTL were negative. Under salt stress, the three QTL, K-1S, K-2S and K-3S were identified. They explain 24% of the phenotypic variation. K-2S has a positive additive effect. Conversely, K-1S and K-3S have negative additive effects.

Sodium-potassium ratio

No QTL were detected under the control regimen. Three QTL were mapped for salt stress: Na/K-1S, Na/K-2S and Na/K-3S on the LGs A3, C9 and C8a. All of the QTL together explain 28% of the phenotypic variation. Na/K-1S was identified at P = 0.05. The additive effect of Na/K-1S and Na/K-2S was negative, while it was positive for Na/K-3S.

Table IV-4: QTL detected with LOD > 1.5 under control treatment (C) for fresh weight, dry weight, SPAD1, SPAD2, relative water content, sodium content Na^+ mg/ g DM and potassium content K^+ mg/ g DM in *Brassica napus* mapping population Mansholts × Samourai. (QTL significant with *P* = 0.05 are marked bold)

Trait	Name of QTL	LG	LOD	Position (cM)	Interval	Flanking Markers	Additive Effect	Phenotypic variation explained (%)
FW	FW-1C	6	1.59	10	8-18	RP1104.H1 -RP428.E1	-0.14	5.49
DW	DW-1C	5	2.01	43	42-46	RP1266.E1 -E3261.2	-0.02	6.86
DW	**DW-2C**	**12**	**5.36**	**74**	**69-77**	**RP1365.H1- CB10026**	**0.40**	**17.3**
DW	DW-3C	13	2.12	173	171-173	RP1365.H3- R1458.H2	0.02	7.25
RWC	**RWC-1C**	**12**	**5.14**	**98**	**91-109**	**WG2D11.E1 -RP1249.H1**	**-0.54**	**16.64**
RWC	RWC-2C	13	2.91	91	80-97	WG5B1.H1 - WG6D6.E1	-0.40	9.8
SPAD1	**SPAD1-1C**	**9**	**4.92**	**72**	**59-81**	**RP1360.E1- RP1253.E1**	**1.2**	**15.99**
SPAD1	**SPAD1-2C**	**12**	**5.6**	**66**	**60-73**	**OPA18.820- RP1365.H1**	**1.19**	**18.01**
SPAD1	SPAD1-3C	16	2.78	57	54-68	CB10278- WG7E10.H2	0.83	9.37
SPAD2	SPAD2-1C	9	2.28	69	58-82	RP1360.E1- RP1253.E1	0.82	7.77
SPAD2	**SPAD2-2C**	**12**	**4.14**	**98**	**93-105**	**WG2D11.E1- RP1249.H1**	**1.10**	**13.65**
K mg / g DM	**K-1C**	**12**	**5.35**	**94**	**85-98**	**OPD20.840- WG2D11.E1**	**-3.18**	**17.53**
K mg/ g DM	K-2C	13	2.16	129	125-130	RP1477.E1 -RP459.H1	-1.87	7.49

- The additive effect is calculated by subtracting Samourai allele by Mansholts allele.
- Interval is the start and end of the genetic distance where the maximal LOD of the QTL were identified.

Table IV-5: QTL detected with LOD > 1.5 under salt treatment (S) for fresh weight, dry weight, SPAD1, SPAD2, relative water content, sodium content Na^+ mg/ g DM and potassium content K^+ mg/ g DM in *Brassica napus* mapping population Mansholts × Samourai. (QTL significant with *P* = 0.05 are marked bold)

Trait	Name of QTL	LG	LOD	Position (cM)	interval	Flanking Markers	Additive Effect	Phenotypic variation explained (%)
FW	FW-1S	13	2.13	152	144-158	OPQ20.780 -OPAG10.63	0.10	7.28
DW	**DW-1S**	**13**	**2.55**	**128**	**113-130**	**RP1477.E1 -RP459.H1**	**0.20**	**8.65**
DW	**DW-2S**	**16**	**2.81**	**44**	**40-51**	**CB10010 -CB10278**	**0.20**	**9.48**
RWC	**RWC-1S**	**13**	**2.5**	**128**	**124-130**	**RP1477.E1 -RP459.H1**	**-0.26**	**8.47**
SPAD1	SPAD1-1S	1	3.55	12	10-15	RP1275.H2 -RP981.H2	-0.77	11.82
SPAD1	SPAD1-2S	9	2.84	29	19-37	RP1175.H1 -TG1H12.E1	0.69	9.58
SPAD1	**SPAD1-3S**	**12**	**4.69**	**68**	**58-74**	**OPA18.820 -RP1365.H1**	**0.92**	**15.32**
SPAD2	SPAD2-1S	1	2.34	15	10-20	RP981.H2 -RP984.H1	-0.66	7.95
SPAD2	SPAD2-2S	3	2.17	101	97-102	CB10271b -W2D5.H1	-0.65	7.41
SPAD2	SPAD2-3S	7	2.49	0	0-8	RP1146.H3 -RP1122.H1	-0.68	8.46
SPAD2	**SPAD2-4S**	**9**	**5.67**	**42**	**30-49**	**TG1H12.E1 -RP1516.E1**	**1.12**	**18.22**
SPAD2	**SPAD2-5S**	**12**	**9.55**	**74**	**71-75**	**RP1365.H1 -CB10026**	**1.48**	**28.71**
SPAD2	SPAD2-6S	13	3.29	0	0-2	E3247.2 -E3348.5	-0.98	10.99
SPAD2	SPAD2-7S	13	3.27	167	160-171	RP1020.H1 -RP1365.H3	-0.82	10.94
Na mg/ g DM	Na-1S	3	4.05	2	0-7	E3347.8 -BRAS002	-2.17	13.37
Na mg/ g DM	Na-2S	19	2.29	2	0-12	E3247.14 -OPAH9.150	-1.77	7.79
K mg/ g DM	K-1S	5	1.88	96	94-114	RP1362.E1 -WG4C5.H1	-1.21	6.45
K mg/ g DM	K-2S	9	3.23	57	42-65	RP1516.E1 -RP1360.E1	1.69	10.82
K mg/ g DM	K-3S	13	2.02	94	88-98	WG6D6.E1 -MR163.2A	-1.25	6.91
Na/K	**Na/K-1S**	**3**	**4.63**	**7**	**1-9**	**BRAS002 -WG4D10.E1**	**-0.04**	**15.14**
Na/K	Na/K-2S	18a	1.81	70	64-72	WG2A11.H1 -RP1144.H1	0.02	6.2
Na/K	Na/K-3S	19	2.16	0	0-8	E3247.14 -OPAH9.150	-0.03	7.36

- The additive effect is calculated by subtracting Samourai allele by Mansholts allele.

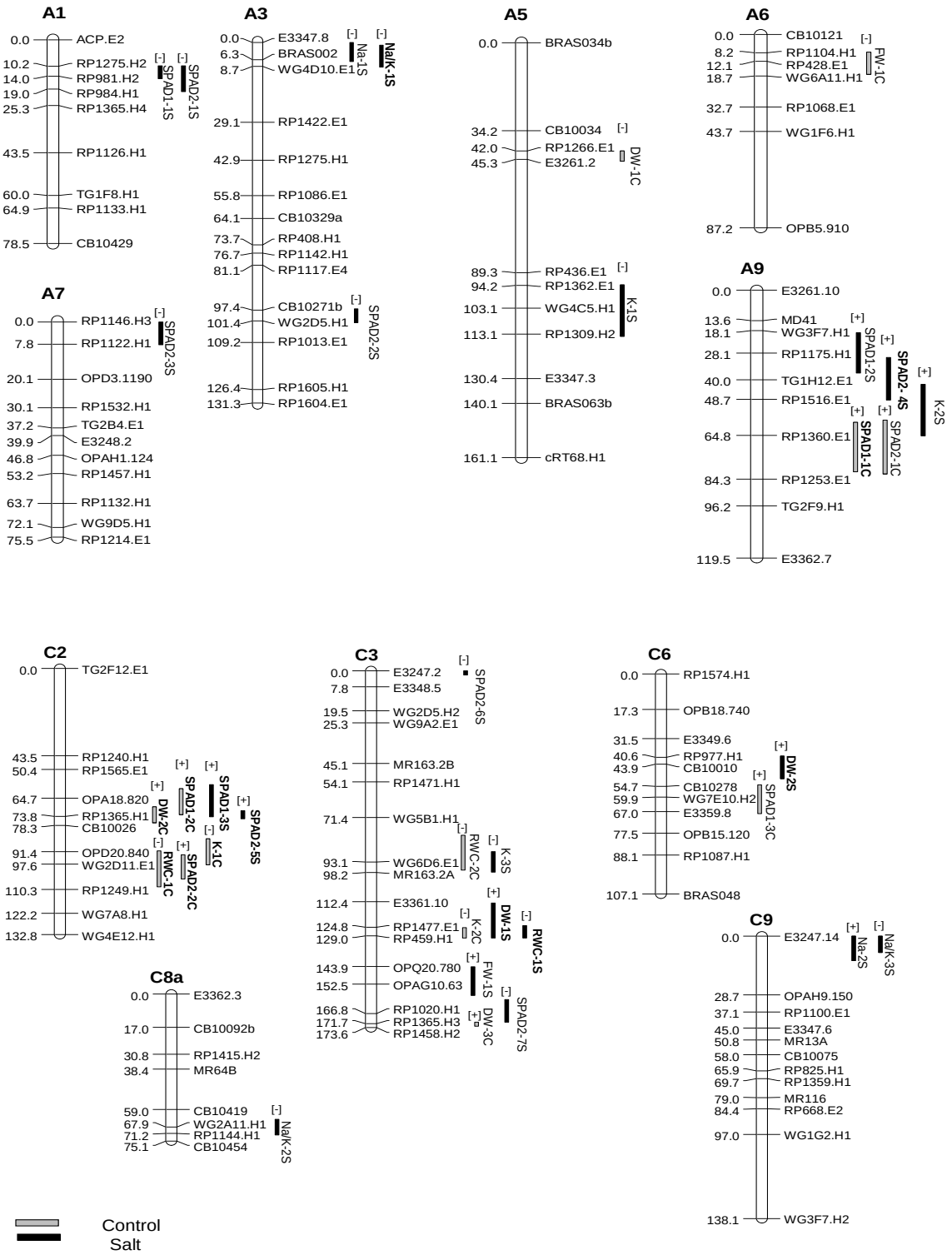


Figure IV-5: Localization of QTL for growth traits in *Brassica napus* DH population Mansholts × Samourai. (QTL significant with *P* = 0.05 are marked bold)

4.5 Discussion I

4.5.1 Traits variations

There was a clear reduction in the FW and DW yield under salt stress compared with the control growth conditions. Also, Liu et al. (2013) reported that 150 mM Nacl reduced dry matter in *Brassica napus* L. cv. Nanyanyou 1. The present study applied a high salt concentration of 200 mM NaCl; hence the lower amount of fresh biomass and dry matter can be attributed to ion-specific toxicity, osmotic stress or a combination of these. Salt stress causes a reduction in leaf area, which consequently reduces the photosynthesis to such a low rate that it is inadequate to sustain growth (Munns 2002; Munns and Tester 2008). In the present study, the water relations parameter RWC showed a slight decline upon salt stress. One plausible explanation for this is that the high concentration of Na^+ and K^+ ions inside the cell ensured sufficient water uptake after all, the RWC showed positive and significant correlations with Na^+ and K^+. Interestingly however, the correlation of RWC with FW and DW was negative under control and stress growth conditions (Tables 2 and 3). This it is more likely that the role of RWC was osmotic adjustment to avoid dehydration rather than blocking the lethal effect of the high dose of Na^+. Similarly, Liu et al. (2013) reported that 150 mM Nacl reduced the RWC in *B. napus* L. cv. Nanyanyou 1.

Under salt stress conditions, there was an increase in the chlorophyll content in the cases of SPAD1 and SAPD2. The high SPAD values for chlorophyll under salt stress are explained in part by anatomical modifications in the leaf area. The reduction of leaf area under salt stress makes the leaf smaller and thicker, which increases chloroplast density per leaf area (Fricke et al. 2004)). Munns et al. (2002) found that in wheat, under 150 mM NaCl, the photosynthesis of the entire plant was reduced, though there was no change in the photosynthesis rate per unit leaf area. The correlation of SPAD1 and SPAD2 with DW was positive and significant, especially under salt stress, which indicates the crucial role of chlorophyll as a photosynthetic pigment for the capture of light energy and hence for carbon assimilation.

Under salt stress, a dramatic increase in Na^+ mg/ g DM was observed it was 210% compared to the control. The correlation between Na^+ and the remaining traits was negative, particularly, with FW, DW and also with K^+. The excessive levels of Na^+ and Cl^- induce nutrient deficiency as a result of the unbalanced ratio between Na^+ and Cl^- and other ions. This nutrient deficiency may be ascribed to a lack of nutrient uptake and an inactivation of the metabolic pathways that are mainly dependent on these nutrients. Additionally, these imbalances cause an unequal distribution and sequestering of the other nutrients inside the plant, leading to a further deficiency of essential nutrients (Grattan and Grieve 1999). The negative and significant correlation between Na^+ and K^+ shows an antagonism between Na^+ ions and K^+. This antagonism is attributed to their similar physiochemical proprieties and atomic size (Shabala and Cuin 2007).

Unexpectedly, in the DH population, K^+ mg/ g DM increased under salt stress by up to 50 mg/ g DM compared to 48 mg/ g DM in the control with an increase of 5%. The

excess K^+ may be used for osmotic adjustment along with the high concentration of Na^+. The explanation for this point was the positive and significant correlation between K^+ and relative water content. Furthermore, the correlation between K^+ and FW and DW was positive and significant, indicating that K^+ promotes plant growth even under salt stress. The capacity to maintain high K^+ is characteristic for salt-tolerant genotypes (Munns 2005). The contribution of K^+ in biochemical and physiological reactions of the cells includes charge equilibration with the negative charges on nucleic acids and proteins, and activation of important enzymes like those involved in pyruvate transport. Moreover, it acts as an osmoticum to ensure turgidity in case of water-deficiency (Maathuis and Amtmann 1999).

4.5.2 QTL analysis and localization

In the present study, we identified associations between different genomic regions and the variations in the measured traits. Several QTL hotspots for many traits were detected. These QTL hotspots for different traits were found on different LGs. For example, the largest hotspot was on LG C3, where QTL for DW, K^+ and RWC were clustered. This clustering is expected as K^+ is involved as an enzyme catalyst in many processes inside the plant cell. Moreover, K^+ acts as an osmoticum. This assumption is supported by the positive and significant correlation with RWC under either control or salt stress. These hotspots exist for these traits suggest two plausible explanations. The first is that the pleiotropic effect depends on one gene residing in this genomic region that controls the variation of these traits. The second is that the effect is due to different, tightly linked genes that control these traits variations independently. Since this hotspot involves three subgroups of QTL, at least three genes reside in these genomic regions. As the additive effects of K-2C and RWC-1S was negative, while the additive effect of DW-1S was positive, these alleles must be in repulsion phase. Another two groups of QTL were found to be proximal to this cluster. The first one was in the middle of LG C3, which includes two QTL for K-3S and RWC-2C. Both of them had negative effects, meaning that the alleles that underlie these traits are in couple phase. It is possible that either one gene with pleiotropic effect or two genes regulate these two traits. The second group was at the bottom of LG C3 and includes two QTL, indicating that they control these traits separately.

On LG C2, other QTL hotspots were observed. One of them involves three QTL, one for each of the traits K^+, SPAD and RWC. The first and the third showed negative additive effect, whilst the second's additive effect was negative, meaning that these alleles are in a repulsion phase. In this region the QTL SPAD2-5S that explains 28% of the phenotypic variance was mapped. It is more than likely that this QTL is a major gene controlling the chlorophyll content under salt stress.

On LG A9, another group of QTL showed interval overlapping. This group includes five QTL. Four of them corresponded to SPAD1 and SPAD2 and one for K^+. The co-segregation of SPAD-related QTL is expected as they are for the same trait at different time intervals. The intervals of SPAD1-2S and SPAD2-4S overlap each other, suggesting that these genomic regions include genes that control chlorophyll content

variation only under salt stress. Conversely, two QTL, SPAD1-1C and SPAD2-1C, revealed overlapping intervals, suggesting that the gene/genes residing in these QTL regulate(s) the chlorophyll trait only under control conditions. The fifth QTL in this hotspot was for K^+, which overlaps with both groups of QTL for SPAD. This overlap might be due to the role of K^+ in protecting chlorophyll from oxidative damage caused by the reactive oxygen species (ROS) induced by salt stress in the chloroplast, which is the site for producing most of ROS.

Of high interest is the co-localization of QTL for Na^+ and Na^+/K^+ on C9. Probably this region harbors a gene or genes coding for plasma membrane or vacuolar antiporters. The plasma membrane antiporter regulates the influx of Na^+ across the plasma membrane. The vacuolar antiporter controls Na^+ compartmentalization in the vacuole to relieve the detrimental effect of excess Na^+ ions in the cytoplasm. For this reason, fine mapping of this region is of great importance in discovering what type of antiporters is hidden here. Additionally, use of the flanking markers to select for lower Na^+ and Na^+/K^+ may help in improving the salt tolerance. The association between Na^+ and Na^+/K^+ on LG C9 is in agreement with the findings of Koyama et al. (2001) in rice, as they found an association between QTL for Na^+ and Na^+/K^+. Also, they found QTL for Na^+ and K^+, which were mapped on different linkage groups, suggesting that these QTL independently control sodium and potassium uptake. The QTL mapped for Na^+ and Na^+/K^+ were clustered together on the LGs A3 and C9 under salt stress only. It shows that these genomic regions are stress-specific, because no QTL were identified for both traits under control.

Our results agree with the findings of Collins et al. (2008), as they have classified the QTL into the two major groups (constitutive QTL) and (adaptive QTL). The constitutive QTL express themselves in a wide spectrum of different growth conditions. The adaptive QTL occur in a limited range of environmental conditions, either control conditions or salinity. An example for constitutive QTL on LG C2 is that the SPAD1-2C and SPAD1-3S were localized together. An example for adaptive QTL on LG A9 is that the SPAD1-1C and SPAD2-1C showed a strong overlap. In tomato, similar results were reported under salinity, where stress-nonspecific QTL were mapped (Foolad et al. 1999). Monforte et al. (1997) identified a major QTL in tomato, which explained 58% of fruit fresh weight under non-stress conditions. Under salt stress, this QTL explain 14% of the variation for the same trait. Regarding the adaptive QTL, our results are in harmony with the findings of Villalta et al. (2007), as they found salt-specific and control-specific QTL for fruit weight in two solanum F7 populations.

In the context of finding the co-localizations of QTL underlying different traits, results similar to ours have been reported. In *B. napus,* Basunanda et al. (2009) found QTL hotspots for some traits linked to heterosis, such as seed germination and seedling growth. This was expected as these traits are developmentally related. Moreover, they found co-localization between traits that seem to be developmentally unrelated, such as biomass yield, plant height and seed yield. In *Arabidopsis,* Ren et al. (2010) found QTL for different growth traits: green seedling as a marker for salt tolerance and root

length sharing the same position on chromosome 1. In barley, Mano and Takeda (1997) mapped QTL for salt tolerance at the germination stage adjacent to the QTL that control the ABA response. Villalta et al. (2007) found co-localization between several QTL that control many traits, such as fruit weight and fruit number, in two solanum F7 population. Siahsar and Narouei (2010) found a co-localization of QTL for different traits in a barley Steptoe x Morex doubled population. They detected a QTL hotspot for RWC, proline content and water soluble carbohydrates. In another study on the same population, a co-localization was observed between QTL for kernel weight and grain protein (Han and Ullric 1994). Ma et al. (2007) identified a cluster of QTL that control related traits such as the salt tolerance index for radicale and plumule.

Conclusion

To sum up, significant reductions in the FW and DW were observed. The remaining traits showed an increase, especially Na^+ mg/ g DM. Unexpectedly, K^+ mg/ g DM showed an increase under salt stress. Consistent with previous studies, several non-stress-specific and stress-specific regions were identified. Both regions are important for selection, as the non-specific genomic regions might give insights into the performance of genotypes under control and salt stress conditions. The adaptive QTL (stress-specific) can be employed for the direct selection of salt-tolerant genotypes. Several QTL hotspots for several traits were identified for related traits like FW and DW, and for traits that seem to be unrelated, such as K^+ and chlorophyll content (SPAD). The co-localization of related and unrelated traits is a great advantage. The markers that are linked to these QTL will be helpful for selection aimed at improving more than one trait.

Because of the complexity of the salt tolerance trait, the polyploid nature of *B. napus* and the large QTL intervals, it was not possible to go further and look for candidate genes behind this variation. Additionally, the genome of *B. napus* is dynamic new homoeologous regions occur after each cross, rendering the positioning of candidate genes therefore very laborious (Wolfgang Ecke, personal communication). To reach this target, further steps are needed, such as testing for the presence of these QTL in different plant materials and fine mapping to precisely verify their position, because no studies have been hereto reported for this trait in *B. napus*.

Altogether, these results are basis from which to explore the salt tolerance of *B. napus*. This goal is closer than ever before, especially after the publication of the full sequences of the *B. rapa* and *B. oleracea,* the corresponding diploid ancestors of *B. napus* and the expected release of the complete sequence of *B. napus* in the next few years.

Part II

Mapping QTL for glucosinolates variation under control and salt stress conditions in *B. napus*

In *B. napus,* very little is known about the genetic control of leaf GSL variation compared to the genetic control of GSL content in seed. Moreover, our knowledge of the effect of salinity on leaf GSL is much more limited due to the scarcity of studies on this issue.

Table IV-6: Systematic and common names of the glucosinolates detected in the leaf

Systematic name	Trivial name	Group	Source	Abbreviation
(2R)2-Hydroxy-3-butenyl	Progoitrin	Aliphatic	Methionine	PRO
3-Butenyl	Gluconapin	Aliphatic	Methionine	GNA
4-Methylsulphinylbutyl	Glucoraphanin	Aliphatic	Methionine	RAA
4-Methylsulphinyl-3-butyl	Glucoraphenin	Aliphatic	Methionine	RAE
4-Pentenyl	Glucobrassicanapin	Aliphatic	Methionine	GBN
2-Hydroxy-4-pentenyl	Napoleiferin	Aliphatic	Methionine	GNL
3-Indolylmethyl	Glucobrassicin	Indolic	Tryptophan	GBC
4-Hydroxy-3-indolylmethyl	4-Hydroxyglucobrassicin	Indolic	Tryptophan	4OH
2-Phenylethyl	Gluconasturtiin	Indolic	Tryptophan	NAS
4-Methoxy-3-indolylmethyl	4-Methoxyglucobrassicin	Indolic	Tryptophan	4ME
N-Methoxy-3-indolmethyl	Neoglucobrassicin	Aromatic	Tyrosine, Phenylalanine	NEO

To make the description of GSL content understandable, it will be described according to three main classes: aliphatic GSL, indolic GSL and aromatic GSL, in both parents and the DH population under control and salt stress conditions.

4.6 Results II

4.6.1 Parents' GSL profile and content under control conditions

We observed wide variations in total leaf glucosinolate content and in the individual glucosinolates between the parental lines Mansholts and Samourai. This was also the case in the DH population. Mansholts showed higher GSL than Samourai under control and salt stress. Noteworthy is that under salt stress Mansholts showed an increase in total GSL due to the increase in aliphatic GSL.

Mansholts GSL content under control conditions

Under control conditions, the total GSL content of Mansholts was 9.59 µmol/ g DM (Table 7). The aliphatic GSL concentration was 7.6 µmol/ g DM, which accounts for 79% of the total GSL (Table 8). The main compositions were PRO, GBN and GNA. The concentrations of these components were 4 µmol/ g DM, 2.10 µmol/ g DM and 1.20 µmol/ g DM, respectively. The proportions of these components in the aliphatic GSL are 53%, 28% and 15%, respectively. Their contributions to the total GSL are 42%, 22% and 12%, respectively. The minor ingredients, GNL, RAA and RAE amount to 0.08 µmol/ g DM, 0.03 µmol/ g DM and 0.18 µmol/ g DM, respectively. Together, they represent 4% of aliphatic GSL and 3% of total GSL (Table 7, and Figures 6a, b, and 7a, b).

The indolic GSL concentration was 1.8 µmol/ g DM, which accounts for 20% of the total GSL (Figure 6a and Table 8). Four components were detected: GBC, NAS, 4OH and 4ME. The concentrations of these were 1 µmol/ g DM, 0.51 µmol/ g DM, 0.17 µmol/ g DM and 0.14 µmol/ g DM, respectively. These components share 55%, 28%, 9% and 8%, respectively of the total indolic GSL. Their contributions to the total GSL are 10%, 5%, 1.6% and 1.4%, respectively (Figures 6a, b and 8a, b, and Table 7).

The aromatic GSL group is represented by NEO with 0.17 µmol/ g DM, with a share of 2% of total GSL (Figures 6a, b and 9, and Tables 7 and 8).

Samourai GSL content under control conditions

Under control conditions, Samourai showed a total GSL content of as high as 6.4 mmol/ g DM. The aliphatic GSL were the predominant class, with 4.25 µmol/ g DM, which accounts for 66% of the total GSL (Table 8). The major components are PRO, GBN and GNA. The absolute values of these components were 1.9 µmol/ g DM, 1.4 µmol/ g DM and 0.67 µmol/ g DM, respectively. The shares of these components in the aliphatic GSL are 46%, 34% and 16%, respectively. Their contributions to the total GSL are 30%, 22% and 10%, respectively (Figure 6a, b, Figure 7a, b). The remaining components of the aliphatic class are GNL, RAA and RAE. Their concentrations are rather low in comparison to the major components. All of them together represent 3% of the aliphatic GSL and 2.8% of the total GSL content (Figures 6a, b and 7a, b, and Table 7).

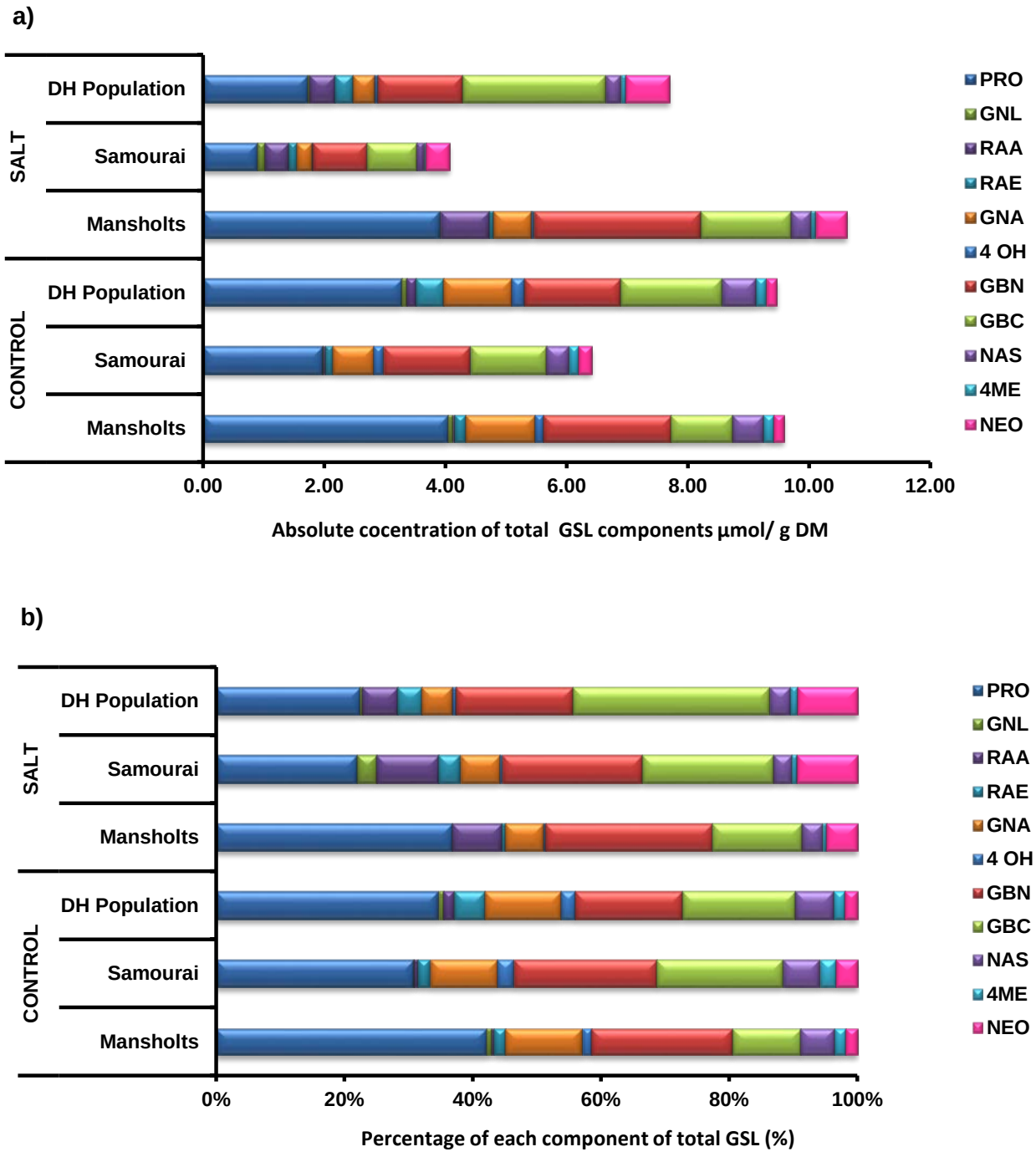


Figure IV-6: (a) Total glucosinolate content µmol/ g DM of each component in parents and the DH population under control and salt, (b) Percentage of each component relative to the total GSL content in parents and the DH population under control and salt

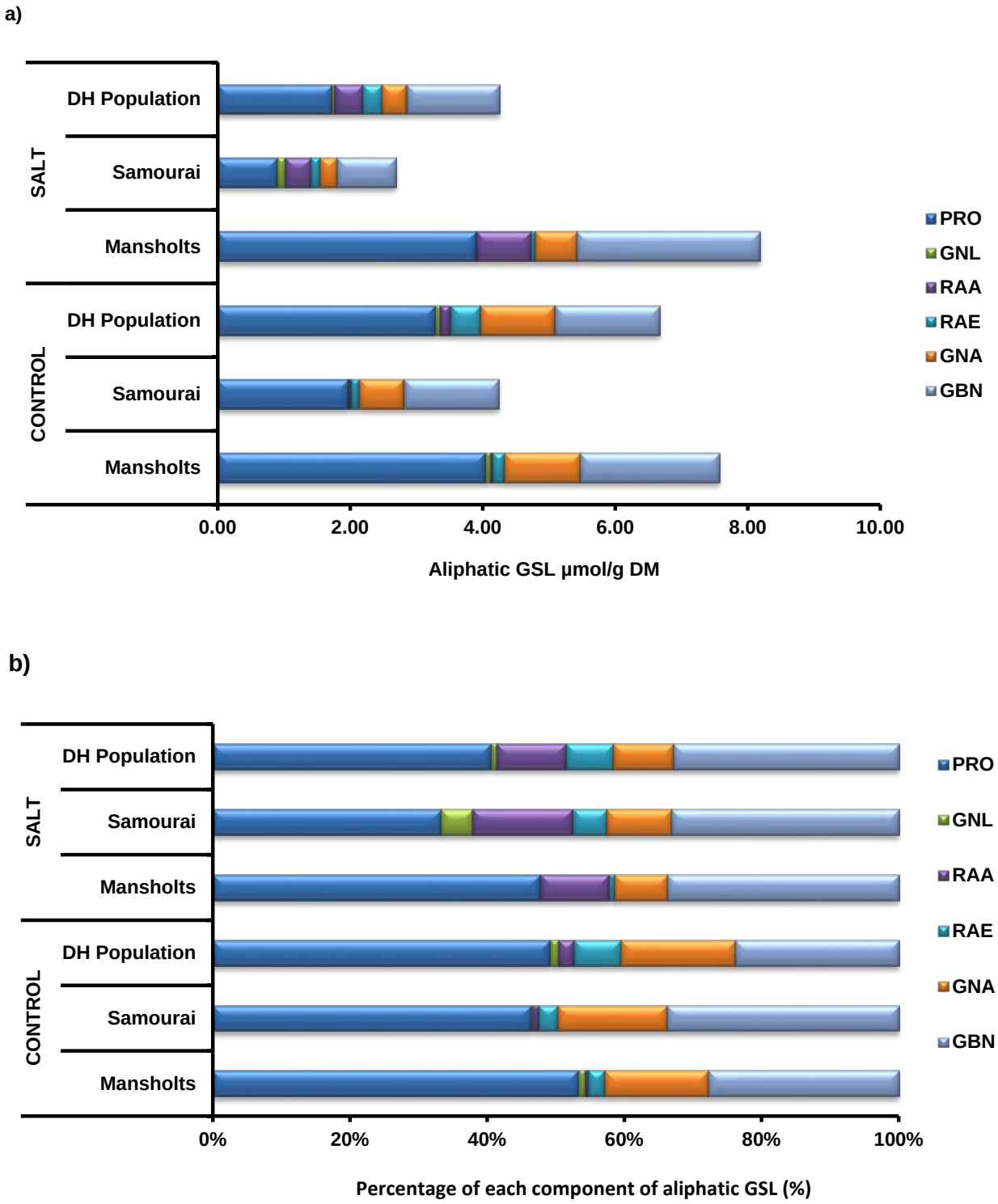


Figure IV-7: a) Absolute values of aliphatic GSL components µmol/ g DM in the parents and the DH population under control and salt, b) Percentage of each ingredient of the aliphatic GSL in the aliphatic GSL content in parents and the DH population under control and salt

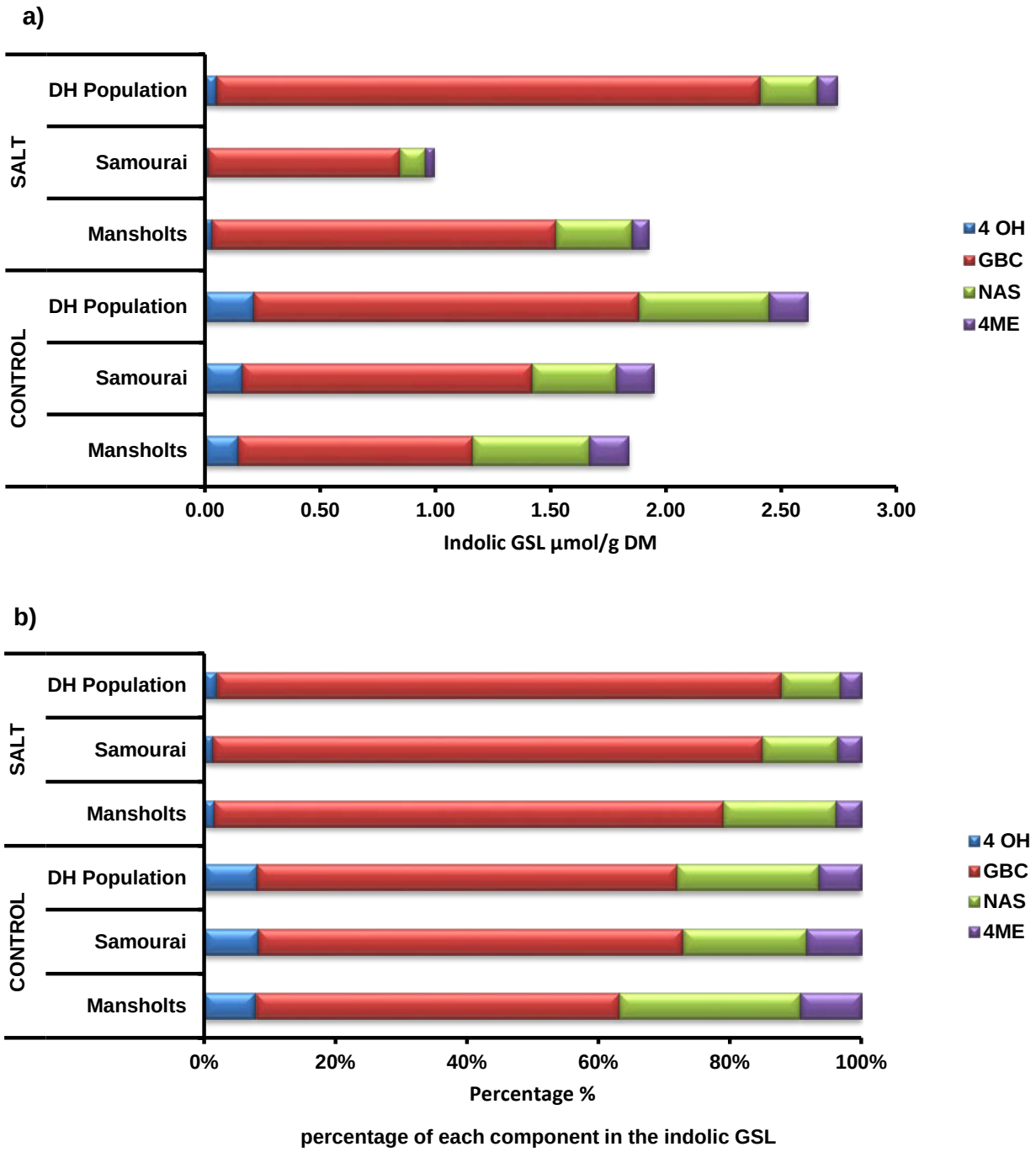


Figure IV-8: a) Absolute values of indolic GSL components µmol/ g DM in the parents and the DH population under control and salt, b) Percentage of each ingredient of indolic GSL in the indolic GSL content in parents and the DH population under control and salt

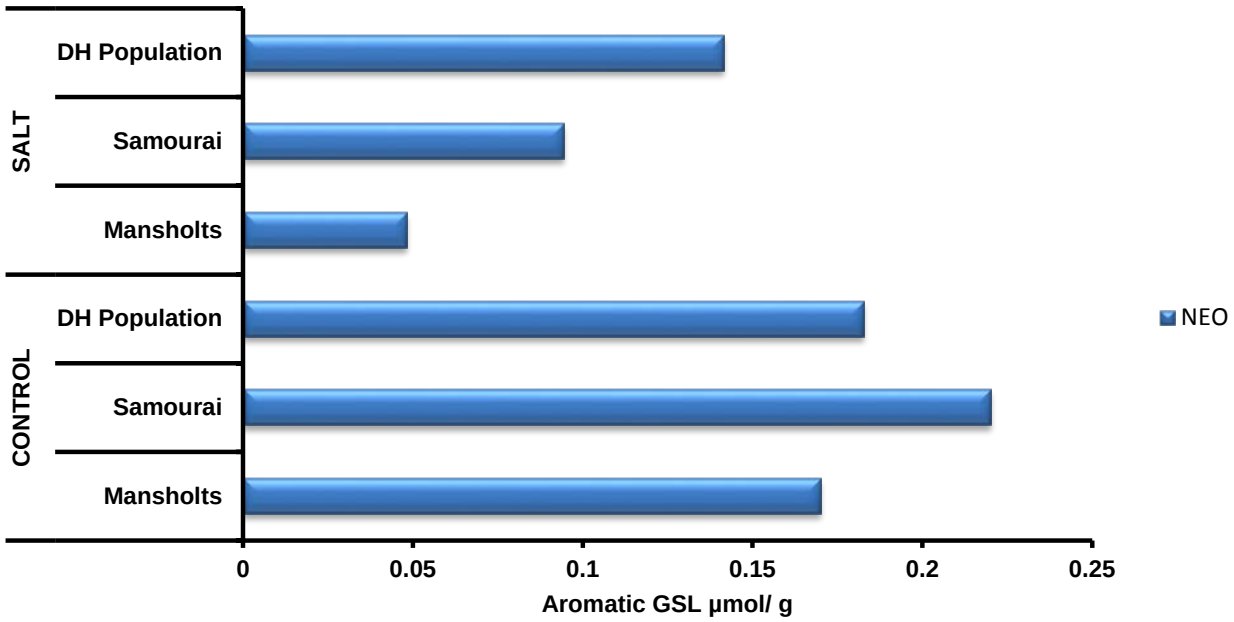


Figure IV-9: Absolute values of aromatic GSL components µmol/ g DM in the parents and the DH population under control and salt

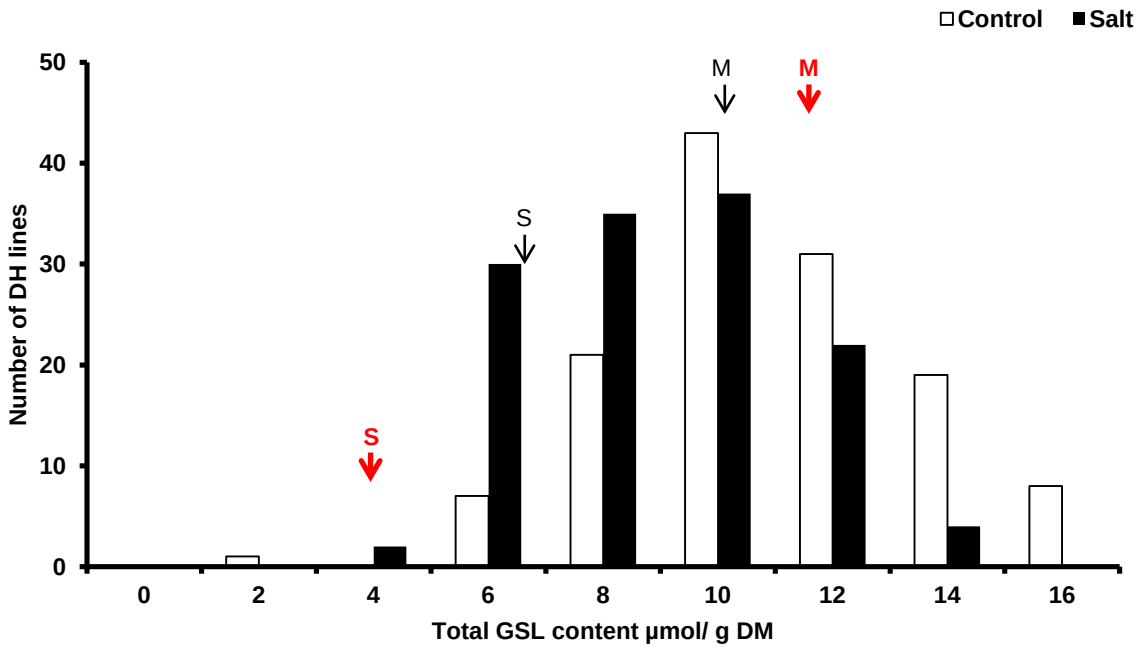


Figure IV-10: Distribution of total GSL µmol/ g DM of *Brassica napus* DH population Mansholts x Samourai under control and salt stress

- Red and bold arrows refers to parents GSL content under salt stress

The indolic GSL represented 30% of the total GSL, with 1.95 µmol/ g DM (Table 8). GBC and NAS are the major components with 1.3 µmol/ g DM and 0.37 µmol/ g DM, respectively; their shares in the indolic GSL are 64% and 19%, respectively. These components share 20% and 60% of the total GSL, respectively. The minor components, 4OH and 4ME have the same concentration of 0.16 µmol/ g DM; collectively they share 16% of the GSL and 3% of the total GSL (Figures 6a, b and 7a, b, and Tables 7 and 8).

The NEO concentration was 0.22 µmol/ g DM, which represents less than 4% of the total GSL (Figure 6a, b, Figure 9, and Table 7).

The ratios of the GSL content of Mansholts relative to Samourai are 151%, 180%, 94% and 77%, and for total GSL, aliphatic, aromatic and indolic GSL, respectively. These findings declare that Mansholts have a high aliphatic GSL content compared to Samourai. Nevertheless, Samourai show high indolic and aromatic GSL. Regarding the individual components, Mansholts dominated Samourai in all GSL component categories, except RAA, 4OH and GBC (Figure 6a, b, and Table 7).

4.6.2 Parents' GSL profiles and content under salt conditions

We observed a modification of the total GSL content in the parental lines under salt stress conditions. Mansholts exhibited an increase in the total GSL, while Samourai showed a reduction in the total GSL content.

Mansholts GSL profile and content under salt

Mansholts' total GSL increased by 9%, from 9.7 µmol/ g DM under control conditions to 10.6 µmol/ g DM under salt stress. The aliphatic GSL increased by 8%, from 7.6 µmol/ g DM under control conditions to 8.2 µmol/ g DM, which equals 77% of total GSL (Tables 7 and 8). GBN increased to 2.8 µmol/ g DM, which comes to 34% of aliphatic GSL and 26% of total GSL. RAA increased to 0.89 µmol/ g DM, representing 10% of aliphatic GSL and 7% of total GSL (Figure 7a, b, and Table 7). The remaining components decreased. PRO and GNA were the major components with 3.9 µmol/ g DM and 0.63 µmol/ g DM, respectively. These concentrations represent 48% and 8% of the aliphatic GSL, respectively. The shares of the major components in the total GSL are 37% and 5%, respectively. RAE was found in small amounts, and GNL completely disappeared.

Indolic GSL increased by 4%, from 1.84 µmol/ g DM to 1.92 µmol/ g DM, thus accounting for 18% of total GSL. GBC increased to 1.5 µmol/ g DM, which was 77% of the total indolic GSL and 14% of the total GSL. The remaining 4OH, NAS and 4ME decreased relative to their concentrations under control conditions. Together they represent 23% of the indolic GSL and less than 3% of the total GSL (Figure 8a, b, and Table 8). Despite the increase in the indolic GSL under salt stress, their contribution to the total GSL fell from 30% under control to 18% under salt stress conditions (Table 8). The aromatic GSL component, NEO, significantly decreased to be 0.5 µmol/ g DM, sharing 5% of the total GSL.

Samourai GSL profile and content under salt stress conditions

Samurai's total GSL content fell by 34%, from 6.4 µmol/ g DM to 4.1 µmol/ g DM. The aliphatic GSL dropped by 37%, from 4.2 µmol/ g DM to 2.7 µmol/ g DM (Figure 6a, b). Nevertheless, the aliphatic GSL represents 66% of the total GSL content (Table 8). RAA, RAE and GNL increased. The highest increase was scored for RAA, which was 0.39 µmol/ g DM, accounting for 15% of aliphatic GSL and 9.5% of total GSL. Similarly, RAE and GNL accreted, but their contributions were much less than the other components, with contributions of 10% and 3% of aliphatic and total GSL, respectively (Figures 6a, b and 7a, b and Table 7). The major aliphatic components, PRO, GBN and GNA, decreased dramatically relative to their concentrations under control. PRO, GNA and GBN were estimated at 0.89 µmol/ g DM, 0.89 µmol/ g DM and 0.25 µmol/ g DM, respectively. The contributions of these three components in the aliphatic GSL were 33%, 33% and 9%, respectively. Their shares in the total GSL were 22%, 22% and 6%, respectively (Figures 6a, b and 7a, b, and Table 7).

The total indolic GSL declined extremely, by up to 49%, from 1.95 µmol/ g DM under control conditions to 0.99 µmol/ g DM under salinity. The indolic GSL contribution to the total GSL content was 24% (Table 8). All ingredients declined: GBC, NAS were quantified as 0.83 µmol/ g DM and 0.11 µmol/ g DM, respectively. The shares of these constituents in the indolic GSL are 83% and 11%, respectively. The contributions of GBC and NAS to the total GSL are 20% and 3%, respectively. The remaining types, 4OH and 4ME were detected in rather small amounts. Their shares represent 6% of the indolic GSL and 2% of the total GSL (Figures 6a, b Figure 8a, b, and Tables 8 and 7).

The aromatic GSL decreased significantly, by 59 % to 0.1 µmol/ g DM, representing the smallest portion of the total GSL, i.e. 10% (Figures 6a, b, and 9 and Table 8).

Under salt stress, the ratio of Mansholts GSL content to Samourai GSL content became much higher compared to the ratio under control. The ratios were 260%, 320%, 98% and 55%, corresponding to the total GSL content, aliphatic GSL content, indolic and aromatic GSL, respectively. Similar to the results under control conditions, Mansholts dominated Samourai in the total GSL content, though Samourai dominated Mansholts in in terms of the indolic and the aromatic GSL content.

4.6.3 Glucosinolate profile and content of the DH population under control conditions

Control versus salt stress conditions produced a significant difference in the total GSL content and the single components (Appendix 10). Under control and salt stress, a transgressive distribution was observed in the DH population for all single components. The main aliphatic, indolic and aromatic groups as well as the total GSL showed a similar pattern (Appendices 10 and 11, and Figure 10). These data reveal that the total GSL content of some DH lines exceeded that of the parent with high GSL content (Mansholts), while others showed GSL content lower than that of the parent with the lower GSL content (Samourai).

A large variation in the total GSL was observed in the DH population under control or salt stress. Under control conditions, it ranged from 1.7 µmol/ g DM to 16 µmol/ g DM with a mean value of 9.8 µmol/ g DM. Under salt treatment, the total GSL content ranged from 2.5 µmol/ g DM to 13.3 µmol/ g DM with a mean of 7.2 µmol/ g DM (Figure 6a, b, and Table 7).

Aliphatic GSL under control conditions

Aliphatic GSL represented the main portion, ranging from 0.63 µmol/ g DM to 13.5 µmol/ g DM with a mean value of 6.7 µmol/ g DM. The share of aliphatic GSL in the total GSL was 70% (Table 8). The major constituents, PRO, GBN and GNA, were found to have the concentrations 3.3 µmol/ g DM, 1.6 µmol/ g DM and 1.1 µmol/ g DM, respectively. The shares of the major components in the aliphatic GSL are 49%, 24% and 11%, respectively. Their contributions to the total GSL are 33%, 17% and 11%, respectively (Figure 6a, b and Table 7). The remaining minor GSL types, RAE, RAA and GNL, were quantified at 0.45 µmol/ g DM, 0.15 µmol/ g DM 0.08 µmol/ g DM, respectively. Together, they represent 10% of the aliphatic GSL and 7% of the total GSL content (Figure 6a, b and Table 7).

Indolic GSL under control conditions

Indolic GSL represented the second largest portion, ranging from 1.6 µmol/ g DM to 4.9 µmol/ g DM, with an average of 2.6 µmol/ g DM. The indolic GSL accounted for 28% of the total GSL (Table 8). The superabundant constituents, GBC and NAS, were detected in the concentrations 1.2 µmol/ g DM and 0.56 µmol/ g DM, respectively. They are 64% and 22% of the indolic GSL, respectively. The shares of GBC and NAs in the total GSL come to 18% and 6%, respectively. The minor constituents, 4OH and 4ME, were detected in 0.21 µmol/ g DM and 0.17 µmol/ g DM, respectively. Collectively, 4OH and 4ME represent 14% of the indolic GSL and 4% of the total GSL (Figures 6a, b, and 8a, b, and Table 8).

Aromatic GSL under control conditions

Only one component NEO was quantified. It varied from 0 µmol/ g DM to 0.48 µmol/ g with a mean of 0.18 µmol/ g DM, and a share of 2% of the total GSL (Figures 6a, b, and 9, and Tables 7 and 8).

4.6.4 Glucosinolate profile and content of the DH population under salt stress

The average total GSL content of the DH population declined by 20% from 9.5 µmol/ g DM under control conditions to 7.7 µmol/ g DM under salt stress (Table 7).

Aliphatic GSL under salt stress

Under salt stress, the aliphatic GSL ranged from 0.33 µmol/ g to 8.59 µmol/ g. They declined by 39%, from 7 µmol/ g DM under control conditions to 4.31 µmol/ g DM under salt stress, representing 60% of total GSL (Table 8). All types showed a dramatic decrease except RAA, which significantly increased to 0.43 µmol/ g DM. The proportion of RAA is 13% of the aliphatic GSL and 6% of the total GSL, respectively. The major components, PRO, GBN and GNA, were found in values of 1.7 µmol/ g DM,

1.4 µmol/ g DM and 0.37 µmol/ g DM, respectively. The shares of PRO, GBN and GNA in the aliphatic GSL are 40%, 32% and 9%, respectively, and their ratios in the total GSL are 24%, 19% and 5%, respectively. The respective concentrations of RAE and GNL are 0.29 µmol/ g DM and 0.04 µmol/ g DM. Together; they represent less than 8% of the aliphatic GSL content and less than 5% of the total GSL content (Figure 6a, b and Table 7). Interestingly, under salt stress the parental lines Mansholts and Samourai and the DH population showed an increase in RAA.

Indolic GSL under salt stress

The indolic GSL increased by 5% from 2.6 µmol/ g DM under control conditions to 2.7 µmol/ g DM under salt stress (Table 8). The minimum and the maximum values were 0.98 µmol/ g DM to 5.88 µmol/ g DM, respectively, with an average of 2.7 µmol/ g DM. The indolic GSL are 38% of the total GSL (Table 8). The concentration of the dominant component GBC is 2.36 µmol/ g DM, which represents 86% of the indolic GSL and 33% of the total GSL. The amounts of NAS, 4OH and 4ME were 0.49, 0.77 and 0.25 respectively. Altogether, the minor components share 14% of the indolic class and 5% of the total GSL content (Figures 6a, b and 8a, b, and Table 7).

Aromatic GSL under salt stress

The single component NEO showed a dramatic increase of 75%. NEO ranged from 0.07 µmol/ g DM to 4.55 µmol/ g DM, with an average of 0.72 µmol/ g DM, which represents 10% of total GSL (Figures 6a, b and 9 and Table 8).

4.6.5 Correlations

Under control conditions, the aliphatic GSL components correlate positively and significantly with each other in most cases; this is expected because they share the same precursor. The correlations of the aliphatic GSL components with the indolic ones were positive. Nevertheless, the correlation between GNA and GBC was negative and significant (r = -0.24**). Similarly, 4OH correlated negatively and significantly with GNA RAA (r = -0.23**). As to the indolic GSL, the correlations were positive and significant between all components except 4OH, which correlated negatively with the remaining indolic ingredients, where the correlation was negative and significant with 4ME (r = -0.18*), (Table 9).

Similarly, under salt stress, the aliphatic components correlated positively with each other. Likewise, the correlations among the different indolic GSL constituents were positive except 4OH, which revealed a weak and negative correlation with GBC and NAS. In the context of correlation between the aliphatic and the indolic GSL components, PRO and GBN exhibited negative correlations with all the indolic GSL components except NAS. Surprisingly, the correlations of NEO as an aromatic GSL with the individuals of the aliphatic and the indolic GSL were negative, especially with the major aliphatic GSL components (Table 10).

Table IV-7: Minimum maximum and mean of GSL content µMol/ g DM of *Brassica napus* mapping population Mansholts × Samourai and parents under control and salt treatment (200 mM NaCl)

Traits	DH population						Mansholts		Samourai	
	Min		Max		Mean		Mean		Mean	
	Control	Salt	Control	Salt	Control	Salt	Control	Salt	Control	Salt
PRO	0.40	0.00	6.56	4.88	3.28	1.73	4.04	3.91	1.97	0.89
GNL	0.00	0.00	0.24	0.78	0.08	0.04	0.08	0.00	0.01	0.12
RAA	0.00	0.00	0.72	1.77	0.15	0.43	0.03	0.82	0.04	0.39
RAE	0.00	0.00	1.03	1.39	0.45	0.29	0.18	0.07	0.12	0.13
GNA	0.00	0.00	4.66	1.87	1.12	0.37	1.15	0.63	0.67	0.25
4OH	0.00	0.00	1.23	0.49	0.21	0.05	0.14	0.03	0.16	0.01
GBN	0.18	0.00	3.62	3.48	1.58	1.40	2.10	2.76	1.44	0.89
GBC	0.24	0.65	3.58	5.72	1.67	2.36	1.02	1.49	1.26	0.83
NAS	0.00	0.00	1.34	0.77	0.56	0.25	0.51	0.33	0.37	0.11
4 ME	0.00	0.00	0.30	0.60	0.17	0.09	0.17	0.07	0.16	0.04
NEO	0.00	0.00	0.48	2.58	0.18	0.72	0.17	0.51	0.22	0.38
SUM	1.71	2.50	16.00	13.33	9.47	7.69	9.59	10.62	6.41	4.06

- C = Control S = Salt

Table IV-8: Minimum, maximum and mean values of the major categories; aliphatic, indolic and aromatic GSL µmol/ g DM of *Brassica napus* mapping population Mansholts × Samourai and parents under control and salt treatment (200 mM NaCl)

Traits	DH population								Mansholts				Samourai			
	Min		Max		Mean		Percentage		Mean		Percentage		Mean		Percentage	
	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S
Aliphatic	0.63	0.33	13.50	8.59	6.67	4.23	70%	55%	7.58	8.18	80%	77%	4.24	2.68	66%	66%
Indolic	1.63	0.98	4.88	5.98	2.62	2.74	28%	35%	1.84	1.62	18%	18%	1.95	0.99	30%	24%
Aromatic	0.00	0.07	0.48	4.55	0.18	0.72	2%	10%	0.17	0.05	2%	5%	0.16	0.38	34%	10%

- C = Control S = Salt

Table IV-9: Spearman's rank correlations of glucosinolates in *Brassica napus* mapping population Mansholts × Samourai under control condition

	PRO	GNL	RAA	RAE	GNA	4OH	GBN	GBC	NAS	4ME	NEO
GNL	0.16										
RAA	0.36**	0.02									
RAE	0.39**	0.06	0.002								
GNA	0.62**	0.10	0.13	0.15							
4OH	0.10	-0.26**	-0.23**	-0.10	0.29**						
GBN	0.75**	0.16	0.43**	0.36**	0.60**	-0.11					
GBC	0.05	0.16	0.20*	0.02	-0.24**	-0.15	0.06				
NAS	0.28**	0.24**	0.54**	0.17*	0.18*	-0.17	0.41**	0.26**			
4ME	0.06	0.29**	0.31**	0.07	-0.13	-0.18*	0.16	0.58**	0.36**		
NEO	0.07	0.25**	0.16	0.03	-0.10	-0.19*	0.10	0.50**	0.18*	0.57**	
SUM	0.84**	0.21*	0.38**	0.37**	0.62**	0.09	0.81**	0.30**	0.49**	0.29**	0.20*

Table IV-10: Spearman's rank correlations of glucosinolates in *Brassica napus* mapping population Mansholts × Samourai under salt stress (200 mM NaCl) condition

	PRO	GNL	RAA	RAE	GNA	4OH	GBN	GBC	NAS	4ME	NEO
GNL	-0.10										
RAA	0.55**	-0.005									
RAE	0.21*	0.04	-0.05								
GNA	0.55**	-0.40	0.47**	0.10							
4OH	-0.10	0.17*	0.12	0.24**	0.05						
GBN	0.64**	-0.06	0.52**	0.05	0.66**	-0.004					

Table 10 continued from page 94

GBC	-0.07	-0.12	0.004	-0.01	-0.24**	-0.003	-0.28**				
NAS	0.46**	-0.06	0.57**	-0.04	0.39**	-0.01	0.49**	-0.07			
4ME	-0.14	-0.06	0.02	0.15	-0.18*	0.26**	-0.29**	0.39**	-0.02		
NEO	-0.19*	-0.08	-0.15	0.05	-0.22**	0.01	-0.29**	0.29**	-0.12	0.37**	
SUM	0.73**	-0.10	0.55**	0.22*	0.45**	0.08	0.52**	0.37**	0.46**	0.19*	0.17

4.6.6 QTL analysis and localization

The QTL mapping produced ten putative QTL under control and 26 QTL under salt stress conditions. The full description of all mapped QTL, flanking markers, additive effects, and positions is shown in Tables 11 and 12. The QTL localizations and their distribution on linkage groups (LGs) are summarized in Figure 11. A positive QTL additive effect means that Mansholts alleles are increasing the GSL content and a negative additive effect means that Samourai alleles are increasing the GSL content.

QTL associated with the aliphatic GSL under control and salt stress conditions

For the aliphatic GSL, 13 QTL were mapped, five QTL under control and eight QTL under salt stress conditions. All of the QTL described in this paragraph show positive additive effects, indicating that the alleles for increasing the corresponding component are inherited from the parent with high GSL content, i.e. Mansholts. In the case of PRO under control conditions, one QTL, PRO-1C, was detected on LG C3, which explains 5% of the phenotypic variation. Under salt stress, two QTL, PRO-1S and PRO-2S, were mapped on LGs A9 and C2, respectively. PRO-1S was mapped at a significance level P = 0.05 and alone explains solely 29% of the phenotypic variation. PRO-2S explains 6% of the phenotypic variation. In the case of GNL, one QTL, GNL-1C, was identified on LG A3 at a significance level of P = 0.05, which explains 6% of the phenotypic variation. No QTL were mapped for GNL under salt stress. Under control conditions, one QTL, RAA-1C, was mapped for RAA on LG C6, which explains 4% of the phenotypic variation. Likewise, under salt stress, one QTL: RAA-1S was detected on LG A9, which accounts for 8% of the phenotypic variation. The QTL RAA-1S was identified at P = 0.05. Regarding GNA, one QTL, GNA-1C, was mapped on LG A9, which explains 9% of the phenotypic variation.

For RAE under control conditions, one QTL, RAE-1C, was mapped on LG C8a, which governs 8% of the variation; this QTL was identified at P = 0.05 with a negative additive effect. No QTL for RAE were detected under salt stress. For GBN under control conditions, one QTL, GBN-1C, was identified on LG A4, explaining 5% of the variation with a negative additive effect. Under salt stress, four QTL GBN-1S, GBN-2S, GBN-3S and GBN-4S were detected on LGs, A8, C2, C5 and C9, respectively. Altogether they account for 42% of the phenotypic variation. The first three QTL exhibit positive additive effects, while GBN-4S show a negative additive effect.

With regard to total aliphatic GSL, two QTL, Aliphatic-1C and Aliphatic-2C, were identified on LGs, i.e. A4 and A5, respectively. They together explain 12% of the observed phenotypic variation. The additive effect of Aliphatic-1C is negative, while the additive effect of Aliphatic-2C is positive. Three QTL were mapped for the total aliphatic GSL content under salt stress. Two QTL, Aliphatic-1S and Aliphatic-2S, were mapped on LG A9 at 34 cM and 134 cM, respectively. The third QTL, Aliphatic-3S, was identified on LG C2. The additive effects of the three QTL are positive. These three QTL explain a large portion of the phenotypic variation of about 44%. The two QTL, Aliphatic-1S and Aliphatic-3S, were mapped at P = 0.05.

QTL associated with the indolic GSL under control and salt stress conditions

Surprisingly, under control conditions, no QTL were mapped for GBC, NAS and 4ME. One QTL, 4OH-1C, was positioned for 4OH on LG A3, which explains 5% of the phenotypic variation, with a negative additive effect. For 4OH under salt stress, two QTL, 4OH-1S and 4OH-2S, were located on LGs C3 and C7, respectively. They collectively explain 17% of the observed phenotypic variation. The additive effects of both QTL were positive. For GBC, three QTL, i.e. GBC-1S, GBC-2S and GBC-3S, were detected on LGs A3, C2 and C7, respectively. These QTL explain 27% of the phenotypic variation; all of them exhibited positive additive effects. In the case of NAS, two QTL, NAS-1S and NAS-2S, were localized adjacent to the same LG C9 at 41 cM and 52 cM, respectively. These two QTL explain in total 25% of the observed phenotypic variation. NAS-1S has a positive effect, while NAS-2S has a negative additive. In the case of 4ME, one QTL, 4ME-1S, was mapped on LG C4, which explains 4% of the phenotypic variation with a negative additive effect. No QTL was identified for the total indolic GSL under control conditions. Under salt stress, three QTL, Indolic-1S, Indolic-2S and Indolic-3S, were detected on LGs A3, C2 and C7, respectively. The QTL Indolic-3S was identified at P = 0.05. The three QTL account for 25% of the phenotypic variation; for all QTL the additive effects were positive.

QTL associated with the aromatic GSL under control and salt conditions

Under control conditions, one QTL, NEO-1C, was mapped on LG A3; this QTL explains 5% of the phenotypic variation, with a negative additive effect. Under salt stress, two QTL, NEO-1S and NEO-2S, were mapped on LGs C2 and C4, respectively. Both QTL together explain 23% of the observed phenotypic variation. NEO-2S was identified at P = 0.05 and alone accounts for 16% of the observed phenotypic variation. The additive effects of both QTL were negative.

QTL associated with the total GSL content under control and salt conditions

Under control conditions, one QTL, SUM-1C, was mapped on LG A9, which explains 4% of the variation, with a positive additive effect. Under salt stress, two QTL; SUM-1S and SUM-2S, were mapped on LGs A3 and A9, respectively. These two QTL explain 34% of the variation of the total GSL content. SUM-1S and SUM-2S show positive additive effects. The QTL SUM-2S was mapped at significance level P = 0.05 and show a remarkable effect, accounting for 27% of the variation.

It is important to mention that the total GSL content data for seed were provided by Dr. Ecke, Department of Crop Sciences, Division of plant Breeding, Georg-August Universität, Göttingen. These data have been published (Uzunova et al. 1995). We have included the data of total seed GSL as a check in the QTL analysis.

Table IV-11: QTl detected at LOD > 1.2 under control treatment (C) for glucosinolates µmol/ g DM in *Brassica napus* mapping population Mansholts × Samourai. (QTL significant with *P* = 0.05 are marked bold)

Trait	Name of QTL	Chrom	LOD	Position (cM)	Intervals	Flanking markers	Additive Effect	Phenotypic variation explained (%)
PRO	PRO-1C	C3	1.3	93	81 -99	WG5B1.H1 -WG6D6.E1	0.24	4.6
GNL	**GNL-1C**	**A3**	**1.5**	**9**	**6 -19**	**WG4D10.E1 -RP1422.E1**	**0.01**	**5.6**
RAA	RAA-1C	C6	1.2	55	47 -60	CB10278 -WG7E10.H2	0.03	3.8
RAE	**RAE-1C**	**C8a**	**2.2**	**72**	**71 -75**	**RP1144.H1 -CB10454**	**-0.06**	**7.9**
4OH	4OH-1C	C3	1.9	0	0 -5	E3247.2 -E3348.5	0.10	7.0
GBN	GBN-1C	A4	1.2	55	53 -61	WG4A4.H1 -RP1235.H2	-0.15	4.5
NEO	NEO-1C	A3	1.3	100	81 -102	CB10271b -WG2D5.H1	-0.02	4.9
Aliphatic	Aliphatic-1C	A4	1.7	54	50-60	WG4A4.H1- RG1235.H2	-0.55	6.3
Aliphatic	Aliphatic-2C	A5	1.7	134	130-146	E3347.3 -BRAS063b	0.58	6.1
SUM	SUM-1C	A9	1.1	115	96 -119	TG2F9.H1 -E3362.7	0.65	4.0
SEED	**SEED-1**	**A9**	**13.9**	**24**	**19-29**	**WG3F7.H1 -RP1175.H1**	**7.96**	**43.5**
SEED	**SEED-2**	**C2**	**2.3**	**111**	**101-121**	**RP1249.H1 -WG7A8.H1**	**2.89**	**9.1**
SEED	**SEED-3**	**C6**	**3.4**	**55**	**54-60**	**CB10278 -WG7E10.H2**	**3.49**	**13.0**
SEED	**SEED-4**	**C9**	**3.9**	**47**	**37-51**	**E3347.6 -MR13A**	**3.79**	**14.8**

- Additive effect was calculated by subtracting Samourai alleles by Mansholts alleles.
- The seed glucosinolates data are provided by Dr. Ecke; the data came from two experiments conducted 1992/1993 as field experiments in Reinshof, Göttingen.

Table IV-12: QTL detected at LOD > 1.2 under salt treatment (S) for glucosinolates µmol/g DM in *Brassica napus* DH population Mansholts × Samourai**.** (QTL significant with *P* = 0.05 are marked bold)

Trait	Name of QTL	LG	LOD	Position (cM)	Intervals	Flanking markers	Additive Effect	Phenotypic variation explained (%)
PRO	**PRO-1S**	**A9**	**9.7**	**19**	**16 -22**	**WG3F7.H1 - RP1175.H1**	**0.60**	**29.1**
PRO	PRO-2S	C2	1.7	122	115- 127	RP1249.H1 - WG7A8.H1	0.21	6.2
RAA	**RAA-1S**	**A9**	**2.3**	**19**	**14 -27**	**W3F7.H1 -RP1175.H1**	**0.07**	**8.1**
GNA	**GNA-1S**	**A9**	**2.6**	**16**	**13 -19**	**MD41 -W3F7.H1**	**0.08**	**9.1**
4OH	4OH-1S	C3	2.3	125	113 -130	RP1477.E1 -RP459.H1	0.20	8.3
4OH	4OH-2S	C7	2.6	91	81 -105	WG6C1.E1 -TG5B2.H1	0.01	9.1
GBN	GBN-1S	A8	2.7	5	1 -7	RP1227.E1 -OPAI2.119	0.22	9.5
GBN	GBN-2S	C2	4.0	107	100 -115	WG2D11.E1 RP1249.H1	0.28	14.0
GBN	GBN-3S	C5	1.8	47	41 -51	OPT9.862 -RP981.H1	0.17	6.7
GBN	GBN-4S	C9	3.4	97	91 -98	RP668.E2 -WG1G2.H1	-0.24	12.1
GBC	GBC-1S	A3	1.9	109	106 -116	WG2D5.H1 -RP1013.E1	0.24	7.1
GBC	GBC-2S	C2	3.4	113	110 -119	RP1249.H1 -WG7A8.H1	-0.34	12.1
GBC	GBC-3S	C7	2.1	67	62 -77	RP318b.E1 -CB10546	0.28	7.7
NAS	NAS-1S	C9	4.9	41	37 -44	RP1100.E1 -E3347.6	0.12	16.4
NAS	NAS-2S	C9	2.6	52	50 -58	MR13A -CB10075	-0.10	9.1
4ME	4ME-1S	C4	1.2	145	130 -147	RP1235.H1 -RP1198.H1	-0.20	4.3
NEO	NEO-1S	C2	1.9	110	101 -117	WG2D11.E1 RP1249.H1	-0.14	6.8
NEO	**NEO-2S**	**C4**	**4.6**	**119**	**116 -123**	**WG4A4.H2 -TG3D1.H1**	**-0.23**	**15.7**
Aliphatic	**Aliphatic-1S**	**A9**	**8.4**	**17**	**13-19**	**MD41 - WG3F7.H1**	**1.30**	**26.8**
Aliphatic	Aliphatic-2 S	A9	1.9	96	91-97	RP1253.E1- TG2F9.H1	0.54	9.1
Aliphatic	**Aliphatic-3S**	**C2**	**2.6**	**106**	**100-111**	**WG2D11.E1- RP1249.H1**	**0.48**	**7.8**
Indolic	Indolic-1 S	A3	1.9	110	105-118	RP1013.E1- RP1605.H1	0.249	7.0
Indolic	Indolic-2 S	C2	3.5	111	110-117	RP1249.H1- WG7A8.H1	-0.34	12.0
Indolic	**Indolic-3 S**	**C7**	**1.7**	**66**	**62-74**	**RP318b.E1- CB10546**	**0.31**	**7.4**
SUM	SUM-1S	A3	2.0	120	109 -131	RP1013.E1 -RP1605.H1	0.57	7.3
SUM	**SUM-2S**	**A9**	**8.4**	**21**	**18 -26**	**WG3F7.H1 -RP1175.H1**	**1.19**	**26.7**

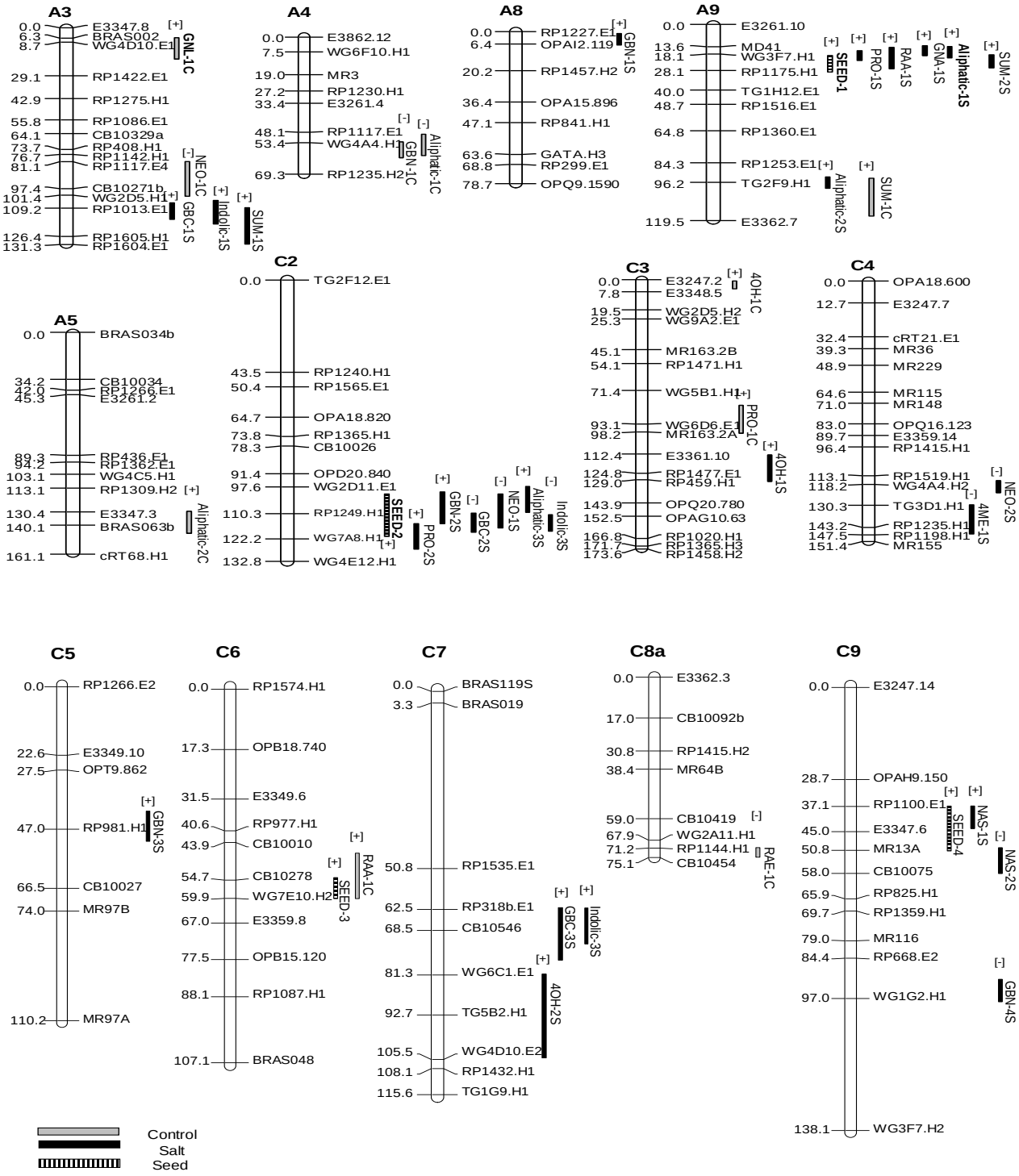


Figure IV-11: Localization of QTL for glucosinolates µMol/g DM in *Brassica napus* DH population Mansholts × Samourai. (QTL significant with *P* = 0.05 are marked bold)

4.7 Discussion II

4.7.1 Variation of GSL under control conditions and salt stress conditions

The aliphatic GSL were predominant in both parental lines and DH populations. Among them, the alkenyl GSL: PRO, GBN and GNA dominated under control and salt conditions. These findings are in harmony with the results of Mithen (1992), who found alkenyl GSL were abundant in *B. napus* leaves, while indolic GSL were the dominant ones in the roots and stem (Mithen 1992). Two aliphatic GSL types are known to be present in lower concentrations, RAA and ALY. Under control conditions, the former was found in a concentration of 0.08 µmol/ g DM, and the second was absent. This finding is consistent with data reported by (Mithen 1992). Under control conditions, the correlation between PRO and its precursor GNA was positive and significant, which can be explained by the high activity of the hydroxylation enzymes in converting GNA to PRO. GNA is derived from RAA through desaturation and loss of the methylsulphinyl moiety by the GS-ALK gene; GNA is hydroxylated by the gene GS-OH to PRO (Li and Quiros 2003). This assumption is supported by taking into consideration that the hydroxylation step under optimal growth conditions is accelerated due to the availability of nitrogen (Yan and Chen 2007). The negative and significant correlation between GNA and GBC might be due to the fact that both are substrates for the hydroxylation enzymes. This negative correlation indicates that there is cross-talk between the biosynthesis cascades of the aliphatic GSL and the indolic GSL. Under salt stress, the correlation of PRO with all the indolic GSL was negative; this is most likely due to the alteration in the GSL biosynthesis to increase the indolic GSL and to decrease the aliphatic GSL. The elevation of GBC under salt stress could be attributed to a decline in the hydroxylation event to produce the hydroxylated indolyl GSL component (McDanell et al. 1988). This hypothesis is supported by the negative correlation between GBC and 4OH.

Under salt stress, the total GSL content of the DH population decreased. Our results agree with the findings of Pang et al. (2012) in the salt-tolerant *Thellungiella halophila,* where the GSL content decreased to under 300 mM NaCl. In contrast to the reduction in total GSL, the indolic GSL exhibited an increase, which agrees with the results of Xin et al. (2008), who found an increase in the total indolic and aromatic GSL in *Arabidopsis* under 150 mM NaCl. Troufflard et al. (2010) stated that in *Arabidopsis,* the indolic GSL increased under K-deficiency growth conditions. Therefore, we can conclude that the increase in the indolic GSL in this population is attributed to K-deficiency, which is a consequence of salt stress. The cross-talk between the biosynthesis of aliphatic and indolic GSL was evident in *Arabidopsis* (Gigolashvili et al. 2009), and in *Brassica rapa* ssp. *Pekinensis* (Kim et al. 2013). These authors reported that several transcription factors belonging to the cytochrome 450 family are involved in the biosynthesis of both the indolic and aliphatic GSL. The reduction or blocking of the biosynthesis of the aliphatic GSL is accompanied by an increase in the indolic GSL and *vice versa* (Grubb and Abel 2006).

Notably, GBC and RAA show an increase, suggesting that they are involved in salt tolerance. In accordance with our results, Guo et al. (2013) observed a 2.1-fold

increase in sulforaphane, which is one of the products of glucoraphanin hydrolysis under 100 mM NaCl in *Brassica oleracea* var. italica cv. Youxiu. The aliphatic GSL RAA possesses three Sulfur atoms. After RAA degradation the released sulfur atoms are responsible for the antioxidant leverage of RAA. Probably, plants tend to accumulate RAA, to hydrolyze it under the sub-optimal growth conditions and to use these three Sulfur atoms to cope with salt stress (reviewed by Falk et al. 2007). Traka et al. (2013) report that in broccoli the accumulation of RAA might due to the steering of assimilates to enrich methionine-derived GSL biosynthesis. The increase in GBC indicates that it acts as an antioxidant. Furthermore, GBC drives the biosynthesis of auxin, which stimulates root growth in *Arabidopsis thaliana* under sub-optimal growth conditions, aiding the plant to look for more nutrients (reviewed by Falk et al. 2007). López-Berenguer et al. (2008) proposed that GSL under salt stress play a role in osmotic adjustment. This conclusion is doubtful because the concentration of GSL is much lower than other the components involved in the osmotic adjustment, such as K^+, N^+ and organic metabolites like proline and glycine betaine. The plausible function of glucosinolates under salt stress might be to overcome the deleterious effects of the reactive oxygen species (ROS) that were generated under salt stress.

Several scenarios have been proposed to explain the reduction in total GSL under salt stress. Under salt stress, plants tend to reduce the biosynthesis of some metabolites, including GSL (Pang et al. 2012, López-Berenguer et al. 2009). This assumption was supported by the findings of Steinbrenner et al. (2012) in *B. rapa,* where they found that temporal stresses like salinity delayed the accumulation of some metabolites, including GSL. Under unfavorable conditions, the biosynthesis of GSL is costly (Textor and Gershenzon 2009). A reduction in GSL under salt stress might result from the leakage of GSL from the vacuole to the cytosol where they are hydrolyzed by myrosinase (Pang et al. 2012). Additionally, it has been reported that GSL are a reservoir for nutrients, like Nitrogen and Sulfur. One of the effects of salinity is to cause nutrient deficiency; therefore, plants hydrolyze GSL and benefit from the released elements as an alternative, so as to ensure the primary stages of metabolism such as protein biosynthesis (reviewed by Martinez-Ballesta et al. 2013).

4.7.2 QTL analysis and localization

In the case of *B. napus,* much less is known about the genetic control of leaf GSL compared to seed glucosinolates. Under control conditions, almost none of the aliphatic-specific QTL and indolic-specific QTL showed intervals of overlapping. This finding suggests that different genomic regions control the biosynthesis of both classes individually. Most likely, the genes that reside in these regions are involved in side chain modification. Two QTL, GNL-1C on LG A3 and GBN-1C on LG A4, were mapped for the aliphatic 5C hydroxylated forms, which suggests the presence of two genes. The first one catalysis the side chain elongation, such as GS-Elong, and the second controls the hydroxylation step, like the GS-OH gene (Li and Quiros 2003, Kliebenstein et al. 2001a). The interval of this QTL GBN-1C overlaps with the QTL for the sum of the aliphatic GSL. This is expected because GBN is one of the major aliphatic components. Two QTL, PRO-1C and GNL-1C, were mapped on LGs: C3 and

A3, respectively, this result shows that two homologous genes are controlling the hydroxylation step one in the A genome and the second in the C genome. In agreement with this, Howell et al. (2003) found that the loci on A9, C2 and C9 were homoeologous loci.

QTL for aliphatic GSL were identified in the A genome on LGs A3, A4, A5. These findings support those of Lou et al. (2008) and Feng et al. (2012), they found QTL for the aliphatic GSL on LGs A3 and A4, however, they used different plant material. These results suggest that the QTL that have been identified in these different studies on these LGs are major QTL that control leaf GSL variation. The QTL for RAA was mapped on LG C6 in the C genome. This is not totally unexpected because it was evident that the members with the C genome have high concentrations of RAA. For total leaf GSL content, one QTL, SUM-1C, was detected on LG A9. Notably, this QTL was mapped at the bottom of the linkage group, while a major QTL for total seed GSL was mapped at the top of this LG. This means the presence a novel locus uniquely controlling the leaf GSL variation.

Twenty-six QTL were identified under salt stress. Of special importance is the presence of two hotspots encompassing 11 QTL: five QTL on A9 and six QTL on C2, where two major QTL for seed GSL were mapped earlier by Uzunova et al. (1995). The hotspot on A9 includes five QTL for different kinds of aliphatic GSL and one QTL for total GSL. This finding suggests that many genes are included in the aliphatic GSL biosynthesis of this genomic region. This co-localization is expected because all of them are methionine-derived GSL. It is likely that this genomic region harbors genes involved in the aliphatic GSL biosynthesis, such as GSL-Elong converting 4-methylthiobutyl into RAA and GS-ALK converting RAA in to GNA after which GNA undergoes a hydroxylation step to PRO (Li and Quiros 2003). The co-localization of QTL for total seed GSL and QTL for leaves on LGs: A9, C2 and C9 is consistent with the results of Harper et al. (2012) in *B. napus.* the gene expression marker (GEM) and SNP leaves associated well with the genes underlying the seed GSL accumulation. Interestingly, of the 11 QTL, five QTL for the aliphatic GSL co-localize with the major QTL for seed GSL on A9 and six QTL coincide with the second major seed GSL QTL on C2, while only one QTL co-localizes with the third major seed GSL on C9. Strong support for our results came from those of Harper et al. (2012), who found that the gene expression marker (GEM) and SNP QTL associated strongly with the previously detected seed QTL on C2, and A9, while the association on C9 was the weakest. These loci may harbor specific genes, transcription factors or enzymes independently regulating the accumulation of methionine-derived GSL. In *Arabidopsis* transcription factors MYB28 (At5g61420), MYB29 (At5g07690) and MYB76 (At5g07700) increase only the accumulation of the aliphatic GSL (Gigolashvili et al. 2007b, Sønderby et al. 2007). The overexpression of these transcription factors is induced by abiotic stressors like wounding (Gigolashvili et al. 2008).

On LG C2, two QTL for kinds other than methionine-derived GSL were clustered with QTL of the aliphatic GSL, which suggests the presence of genes that induce the

biosynthesis of indolic and aromatic GSL. The more plausible explanation is that the salt stress reduces the biosynthesis of aliphatic GSL and induces the biosynthesis of indolic and aromatic GSL. There is evidence that in *B. napus* leaf treatment with Jasmonic acid (JA) or methyljasmonate (MeJA) strongly induced the biosynthesis of indolic GSL (Bodnaryk 1994). Mikkelsen et al. (2003) demonstrated in *Arabidopsis* that indolic GSL increased 3- to 4-fold after treatment with methyljasmonate (MeJA) or after wounding. This elevation in the indolic GSL was owing to the overexpression of the genes CYP79B2 and CYP79B3, which regulate the indolyl GSL biosynthesis. Most likely, the genes in these genomic are involved in the GSL core structure formation. Otherwise, these regions harbor an orthologous of the gene, At1g18570, which encodes the transcription factor HIG1 (high indolic glucosinolates1), the over expression of this gene increased indolyl GSL biosynthesis and reduced the levels of aliphatic GSL in *Arabidopsis thaliana* (Gigolashvili et al. 2007b).

The additive effects of the QTL, which were mapped earlier for seed total GSL and leaf aliphatic GSL on A9, were positive, suggesting that the alleles that increase seed and leaf GSL are inherited from Mansholts, the parent with the high seed and leaf GSL content. On LG C2 the additive effect of QTL for indolic and aromatic QTL was negative, while QTL for the aliphatic GSL showed positive additive effects. This means the alleles for increasing aliphatic GSL were inherited from Mansholts and alleles for indolyl GSL were passed on by Samourai. The increase in the indolic and aromatic GSL may be attributed to the decoding of some genes that regulate the rate of indolic and aromatic GSL biosynthesis. One of them is the gene *CYP83B1,* which catalyzes the transformation of aromatic and indolic aldoximes into their corresponding GSL class (reviewed by Halkier and Gershenzon 2006).

Conclusion

Worth mentioning is that under salt stress, the two GSL type, RAA and GBC, which are known as antioxidants, showed significant increases. Based on these outcomes, our results are in harmony with several results that were reported earlier. The reduction in total GSL in the DH population may be added to a reduction in the biosynthesis of several secondary products, among them GSL degradation by myrosinase or GSL redistribution. Interestingly, the major two QTL hotspots include the major two QTL for seed GSL and the QTL for leaf GSL. On A9, all QTL that were co-localized with the QTL for seed GSL were mapped for aliphatic GSL. The second hotspot on C2 includes QTL for aliphatic and indolic GSL, suggesting an intercross between the accumulations of both classes. The impact of salinity on GSL biosynthesis and accumulation is still an open question. Further work on leaf GSL content is of great importance to come to a comprehensive understanding of the genetic and metabolic mechanisms that lie behind the variation of GSL under salt stress

Chapter V

Mapping QTL for salt tolerance at the young plant stage and leaf glucosinolates in a *Brassica oleracea* DH population

5.1 Introduction

Brassica oleracea is a diploid plant species (2n = 18) and is one of the major edible vegetable crops worldwide. There are mainly six prominent groups. Kales (var. acephala) include green kale, marrow stem kale and collards. Other forms are widely used as edible forage. Cabbages (var. capitata, var. sabauda, var. bullata) include headed cabbages, brussel sprouts, savoy cabbage, and others, as well as kohlrabi (var. gongylodes). Inflorescence kales (var. botrytis, var. italica) include cauliflower, broccoli, sprouting broccoli, and others, as well as branching bush kales (var. fruticosa) and Chinese kale (*B. alboglabra*), which are used as leafy vegetables (Rakow 2004). The large number of morphotypes developed by human selection reflects their malleability to be differentiated into distinctive crops in their growth habits and morphological features (Branca and Careta 2011). In the context of salt tolerance, broccoli and cauliflower were ranked as moderately salt tolerant, while cabbage and brussels sprouts were classified as moderately salt-sensitive (reviewed by Shannon and Grieve 1998). *Brassica oleracea* was classified as salt-sensitive compared with *B. rapa* and *B. napus* (Ashraf et al. 2001). The salt tolerance of *Brassica oleracea* var. *capitata* cultivar 'Golden Acre' was improved significantly by metabolic engineering via transformation with a bacterial *bet*A gene (Bhattacharya et al. 2004).

Brassica oleracea has a distinctive glucosinolate profile with significant quantities of methylthioalkyl and methylsulphinylalkyl. This seems to be plentiful within the cultivated forms of *B. oleracea* (Magrath et al 1993). López-Berenguer et al. (2008) hypothesized that the accumulation of GSL in *B. oleracea* under salt stress plays a role in the osmotic adjustment. Likewise, Guo et al. (2013) observed a 2.1-fold increase in sulforaphane under 100 mM NaCl in *Brassica oleracea* var. italica cv. Youxiu.

5.2 The objectives of this chapter are

1. To assess the effect of salinity on growth at the young plant stage in a *B. oleracea,* and to map the QTL that control salt tolerance.

2. To study the variation in leaf glucosinolate under control and salt stress conditions and to identify the QTL underlying these.

Part I

Mapping QTL for salt tolerance at the young plant stage in *B. oleracea*

5.3 Materials and methods

5.3.1 Plant material

A doubled-haploid (DH) population of *Brassica oleracea* Bo1TBDH of 138 DH lines was tested in the greenhouse. This population was derived from a F1 individual, which was in turn derived by crossing a DH rapid cycling TO1000DH3 line and a DH broccoli line Early Big (Iñiguez-Luy et al. 2009). The full description of this population is summarized in Chapter 3 (3.3.1).

5.3.2 Greenhouse experiment

The parental lines plus 138 DH lines were tested in the greenhouse at the Department of Crop Sciences, Division of Plant Breeding, Georg-August Universität, Göttingen. The first replicate was sown December 3, 2012 and harvested January 25, 2013. The second replicate started on January 28, 2013 and harvested on March 4, 2013. The experimental design and salt application method are described in details in Chapter 4 (4.1). The soil mixture, number of seeds per pot, number of pots for each genotype and lightning regime are also described in Chapter 4 (4.2). The fluctuation in temperature for each replicate and the mean of temperature over the time course of the two replicates are in Appendix 7. The average light intensities were 197 µmol/m2*s for experiment one and 260 µmol/m2*s for experiment two. The light intensity was measured with Sunscan SS1 (Delta-T, England). The relative humidity was 35%. The salt treatment was started on day 25, with 50 mM NaCl for the first application and increased to a final concentration of 100 mM NaCl on day 30. Seven genotypes were placed randomly on each table as checks. The traits measured and the methods used are given in Chapter 4 (3.2). The chlorophyll content was measured by a SPAD-meter Minolta 502 (Osaka, Japan). The leaf GSL content analysis is described in detail in Chapter 4 (4.4). For the statistical analysis, the values of each trait were adjusted as described above and the software and model are described in Chapter 4 (4.5).

Trait abbreviations

Fresh weight (g) = FW
Dry weight (g) = DW
Relative water content = RWC
Chlorophyll content measured by SPAD1 and SPAD2
Sodium content (mg/ g DM) Na^+
Potassium content (mg/ g DM) K^+
Sodium /Potassium ratio Na^+/K^+
Dry matter DM

Parental lines names abbreviations in figures;

EB = Early Big TO = TO1000DH

5.4 Results I

5.4.1 Traits variations

The fresh weight (FW), dry weight (DW) relative water content (RWC), chlorophyll content were measured by (SPAD), sodium content (Na^+ mg/ g DM), potassium content (K^+ mg/ g DM) and sodium/potassium ratio (Na^+/K^+) were recorded. The parental lines show little variation for the traits fresh biomass and dry biomass under both control and salt treatments (Appendix 7). In contrast, a large genetic variation was observed among the DH lines. The genotypic variation, heritabilities calculated from the analysis of variance (ANOVA) for all traits under control and salt treatment are summarized in Table 1. The minimum and maximum ranges of the above mentioned traits and mean values are given in Appendix 5. A transgressive segregation was observed for all treats under both growing conditions (Figures 1, 2, 3 and 4)

Fresh weight

Under control conditions, 86 (62%) of the DH lines exhibited high FW compared to Early Big, the parent with the higher FW. On the other hand, 24 DH lines (17%) produced a lower FW than TO1000DH3, the parent with low FW. The range of FW varied from 2.7 g to 6.8 g. Under salt treatment, most of the DH lines (112 = 81%) showed better performance than Early Big (Figure 1a, b). Only nine DH lines (7%) produced less fresh biomass than TO1000DH3, the parent with low FW. The minimum and the maximum values were 0.96 g and 3.7 g, respectively.

Dry weight

Under control conditions, out of 138 DH lines, 17 (12%) showed lower DW compared with TO1000DH3, the parent with low DW, while 90 DH lines (65%) displayed DW higher than Early Big, the superior parent. The DW values varied from 0.11 g to 0.58 g. Likewise, under salt stress, few genotypes showed a DW lower than the weak parent, whereas five DH lines (4%) produced a DW less than TO1000DH3. A large set comprises 115 DH lines (83%) produced DW higher than Early Big. The minimum and maximum values were 0.11 g and 0.35 g, respectively (Figure 1c, d).

Relative water content

Under control conditions, a set of 27 DH lines (20%) revealed RWC lower than TO1000DH3, the parent with low RWC, whereas 69 DH lines (50%) showed RWC higher than Early Big, the parent with high RWC. The DH population values ranged from 87 to 95. Under salt treatment, RWC was lower than TO1000DH3 in only nine DH lines and higher than Early Big in 112 lines (81%) (Figure 2a, b).

Chlorophyll content measured by SPAD

Figure 3a, b shows the segregation under control conditions, where 27 DH lines (20%) had a chlorophyll content lower than TO1000DH3, the parent with low SPAD values. A large set of DH lines (74 = 52%) exhibit SPAD values higher than Early Big, the parent with the high SPAD values. The lowest value was 34 and the highest 62. Under salt stress, 12 DH lines (9%) exhibited SPAD values lower than TO1000DH3, while 72 DH

lines (52%) showed SPAD values higher than Early Big (Figure 3a, b); the average ranged from 34 to 67.

Sodium content

A dramatic increase in the Na^+ content under salt stress was observed relative to the Na^+ content under control. Under control conditions, 59 DH lines (43%) showed a lower Na^+ content than Early Big, the parent with the lower Na^+ content, while, 67 DH lines (49%) revealed a higher Na^+ content than TO1000DH3, the parent with the high Na^+ content. The range was between 1.43 Na^+ mg/ g DM and 4.44 Na^+ mg/ g DM. Under salinity stress, TO1000DH3 showed Na^+ content higher than Early Big. A group of 14 DH lines (10%) exhibited a Na^+ content lower than Early Big and larger group of DH lines 73 (53%) had sodium content higher than TO1000DH3 (Figure 4a, b). The lowest and the highest values were 17.5 mg/ g DM and 54.3 mg/ g, respectively.

Potassium content

Out of the 138 DH lines, four genotypes (3%) under control conditions showed a K^+ content lower than Early Big, the parent with the low K^+ content, while, 77 DH lines (56%) had a K^+ content higher than TO1000DH3, the parent with the high K^+ content,. The values ranged from 57.67 mg/ g DM to 81.61 mg/ g DM. Surprisingly, under salt stress, the K^+ content of TO1000DH3 was higher than Early Big. A large set encompassing 115 DH lines (83%) showed a lower K^+ content than Early Big. At the same time, a small set of nine DH lines (7%) exhibited a higher K^+ content than to TO1000DH3 (Figure 4c, d). The minimum and maximum values were 22.3 mg/ g DM to 59.61 mg/ g DM.

Sodium and potassium ratio

Under control conditions, 64 DH lines (46%) had a lower Na^+/K^+ ratio than Early Big, the parent with the low Na^+/K^+ ratio, and 40 genotypes (28%) showed a higher Na^+/K^+ ratio than TO1000DH3, the parent with high Na^+/K^+. The minimum and maximum values lie between 0.03 and 0.06. Under salinity stress, only 3 (2%) DH lines showed a lower Na^+/K^+ than Early Big. A large set of 105 DH lines (76%) showed a Na^+/K^+ ratio higher than TO1000DH3 (Figure 4e, f). The values ranged from 0.31 to 1.26.

5.4.2 Correlations

The related traits, such as FW and DW or Na^+ content and Na^+/K^+ ratio show significant positive correlations with each other. There is a positive correlation between FW and DW under control (r = 0.85**) and salt (r = 0.83**) conditions. Likewise, Na^+ content and Na^+/K^+ are positively correlated under control and salt conditions, with (r = 0.89**) and (r = 0.87**), respectively (Tables 2 and 3). The correlations of FW and DW with Na^+ content and Na^+/K^+ are negative and insignificant under control conditions, whereas a significant negative correlation can be observed under salt (Tables 2 and 3). There are significant positive correlations between RWC and all traits under control and salt conditions, except with DW and SPAD. Under control and salt stress, the correlation of SPAD with the remaining traits is negative,

except for FW and DW, which is positive (Table 2 and 3). A significant positive correlation can be observed between K^+ content and all traits except RWC, Na^+ content and the Na^+/K^+ ratio, which is negative and significant under control as well as salt stress (Tables 2 and 3).

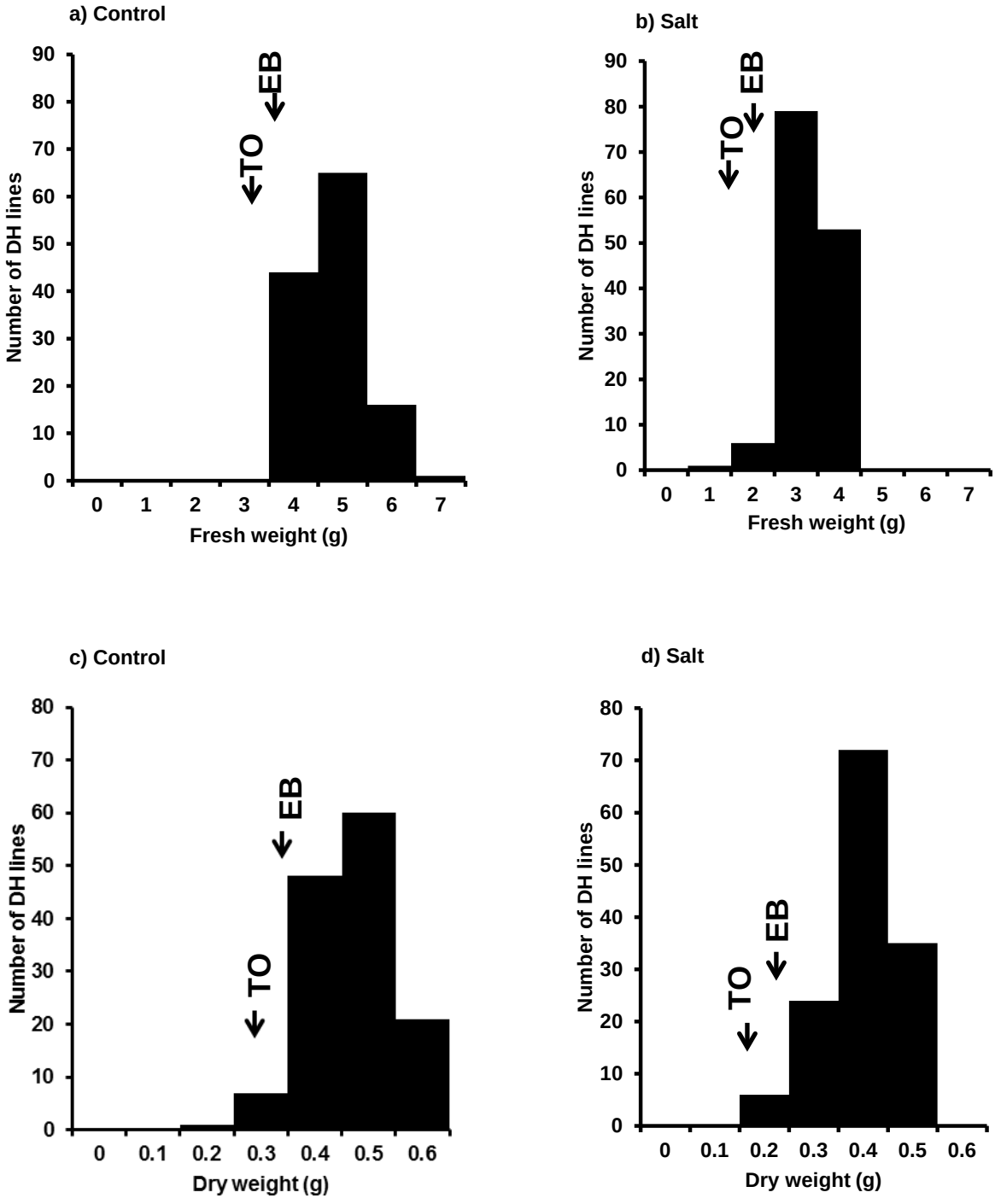


Figure V-1: Frequency distribution of fresh weight and dry weight of *Brassica oleracea* Bo1TBDH population a) Fresh weight under control, b) Fresh weight under salt stress, c) Dry weight under control and d) Dry weight at salt stress

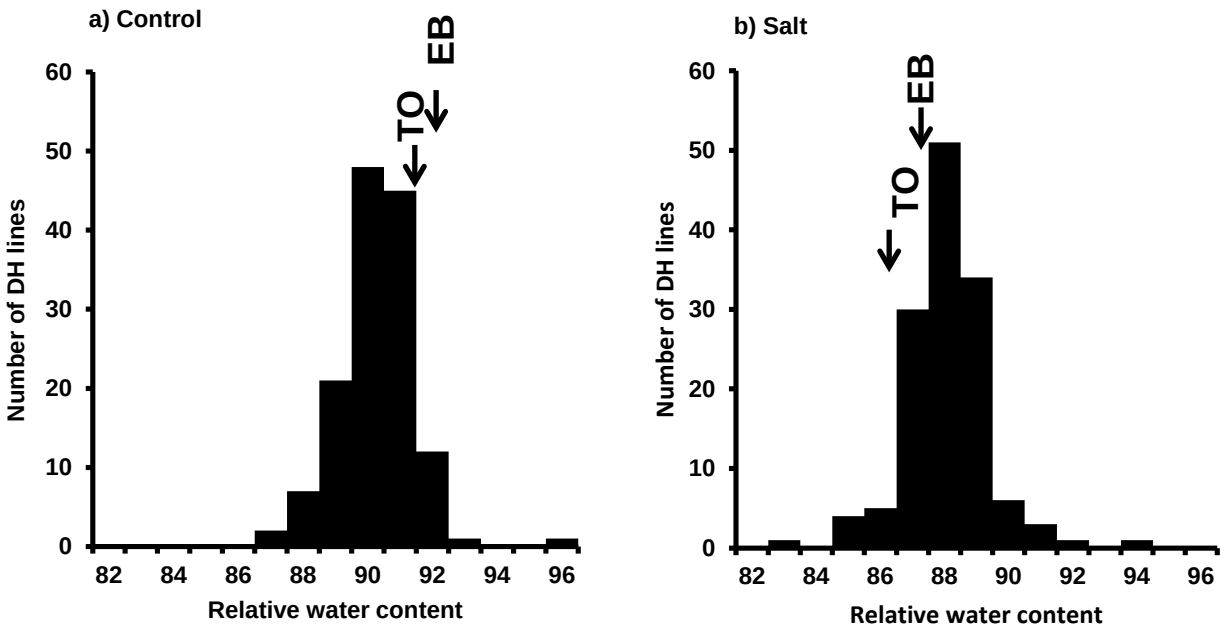


Figure V-2: Frequency distribution of relative water content of *Brassica oleracea* Bo1TBDH population a) Relative water content under control and b) Relative water

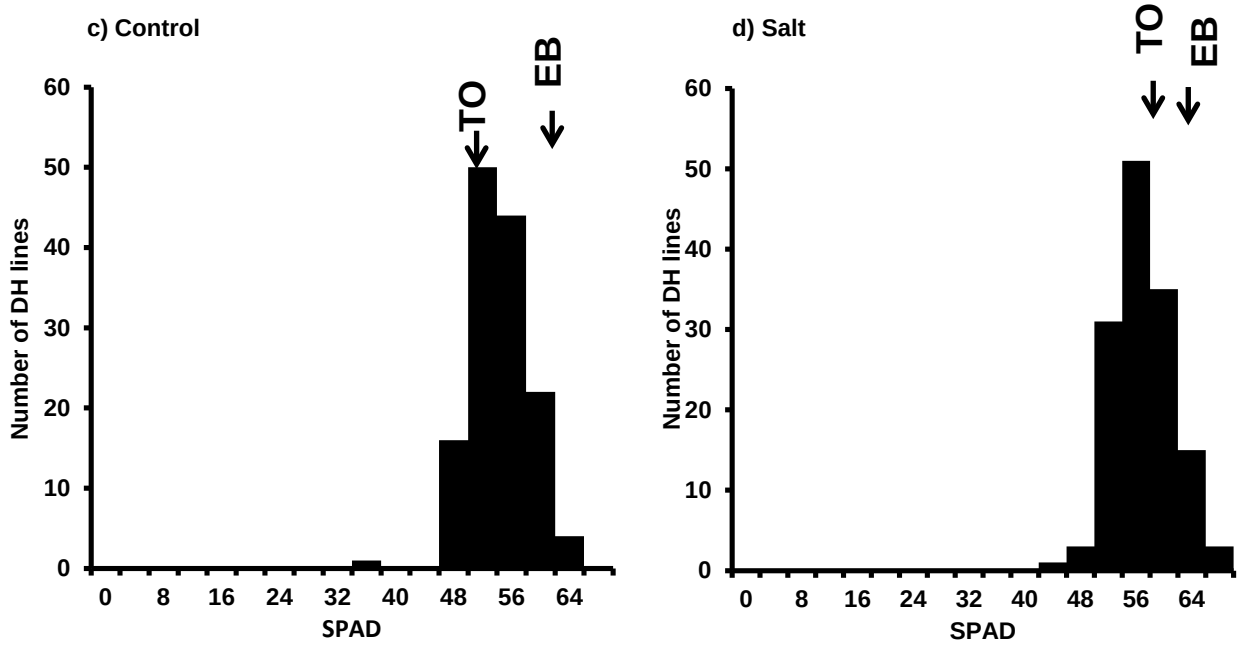


Figure V-3: Frequency distribution of chlorophyll content measured by SPAD of *Brassica oleracea* Bo1TBDH population a) SPAD under control and b) SPAD under salt stress

Table V-1: Mean squares, respective F tests, and heritabilities estimated from the ANOVA of *Brassica oleracea* mapping population Bo1TBDH, 138 DH lines were tested under control conditions and at salt treatment (100 mM NaCl)

	Control				Salt			
Sources of Variance	**Genotypes (G)**	**Replicates (R)**	**G ×R**	**h^2**	**Genotypes (G)**	**Replicates (R)**	**G ×R**	**h^2**
DF	137	1	137		137	1	137	
FW(g)	1.32**	5.31**	0.28	78.52	0.59**	11.45**	0.17**	70.90
DW(g)	0.01**	0.021*	0.004	70.6	0.010**	0.003**	0.003	71.37
RWC	36.56**	388.33**	9.126	75.04	36.25**	372.17**	6.90	80.95
SPAD1	2.47**	54.90**	1.1632	52.96	6.48**	185.34**	3.97	38.75
Na^+ mg/ g DM	0.26	-	-	-	35.5	-	-	-
K^+ mg/ g DM	60.90	-	-	-	43.6	-	-	-
Na^+/ K^+	0.001	-	-	-	0.03	-	-	-

- ** Significant at *P* = 0.01; * significant at *P* = 0.05 and + significant at *P* = 0.1
- For Sodium content (Na^+ mg/ g DM), Potassium content (K^+ mg/ g DM) and Sodium /Potassium ratio (Na^+/ K^+) the dry matter of each genotype of replicate one was combined with replicate two, therefore there were no replication and no heritability was calculated for these traits.

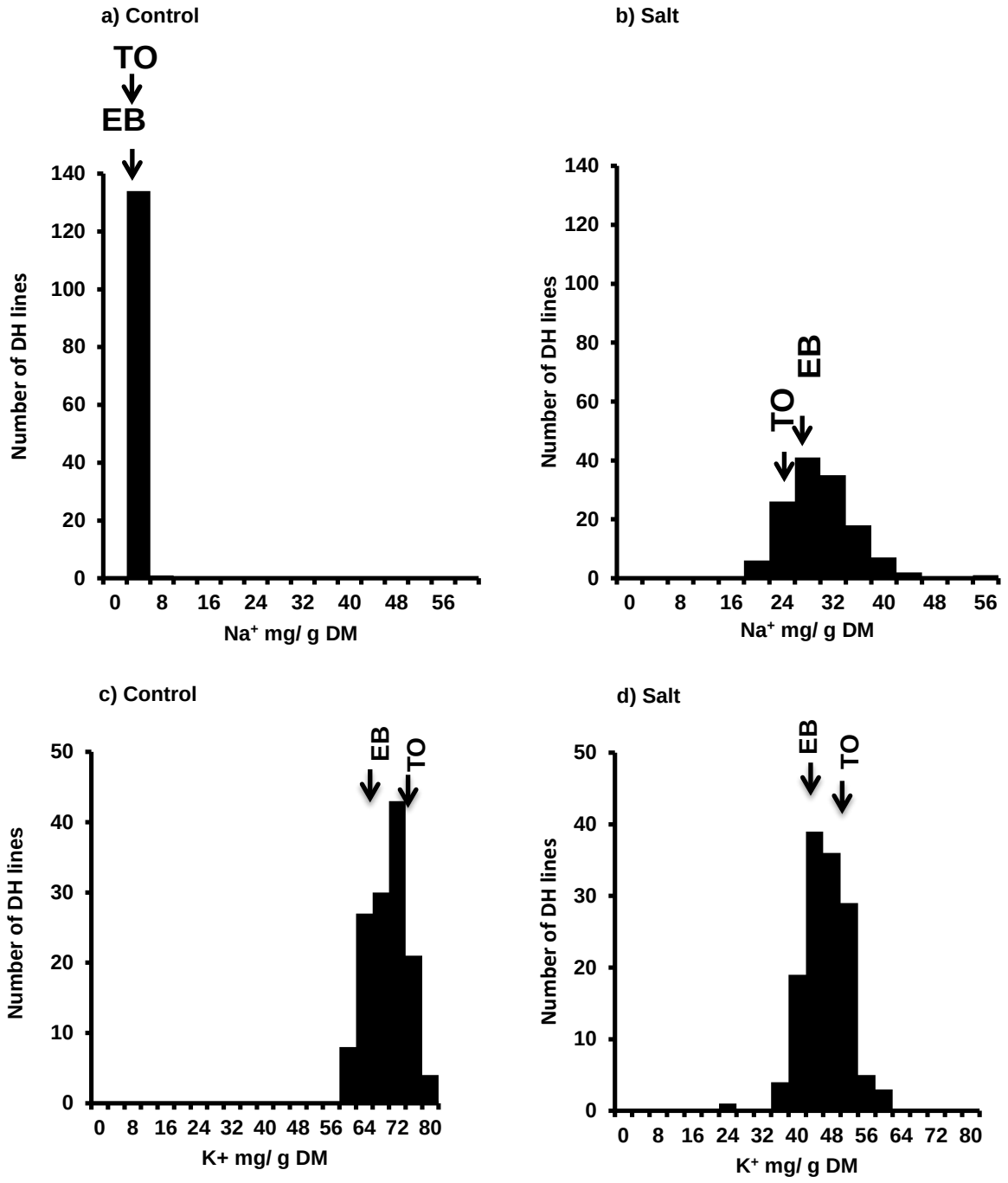


Figure V-4: Frequency distribution of sodium content Na^+ mg /g DM and potassium content K^+ mg/ g DM of *Brassica oleracea* Bo1TBDH population a) Sodium content under control, b) Sodium content under salt stress , C) Potassium content under control, d) Potassium content under salt stress, e) Na^+/K^+ ratio under control and f) Na/K ratio under salt stress

Figure V-4 continued from page 112

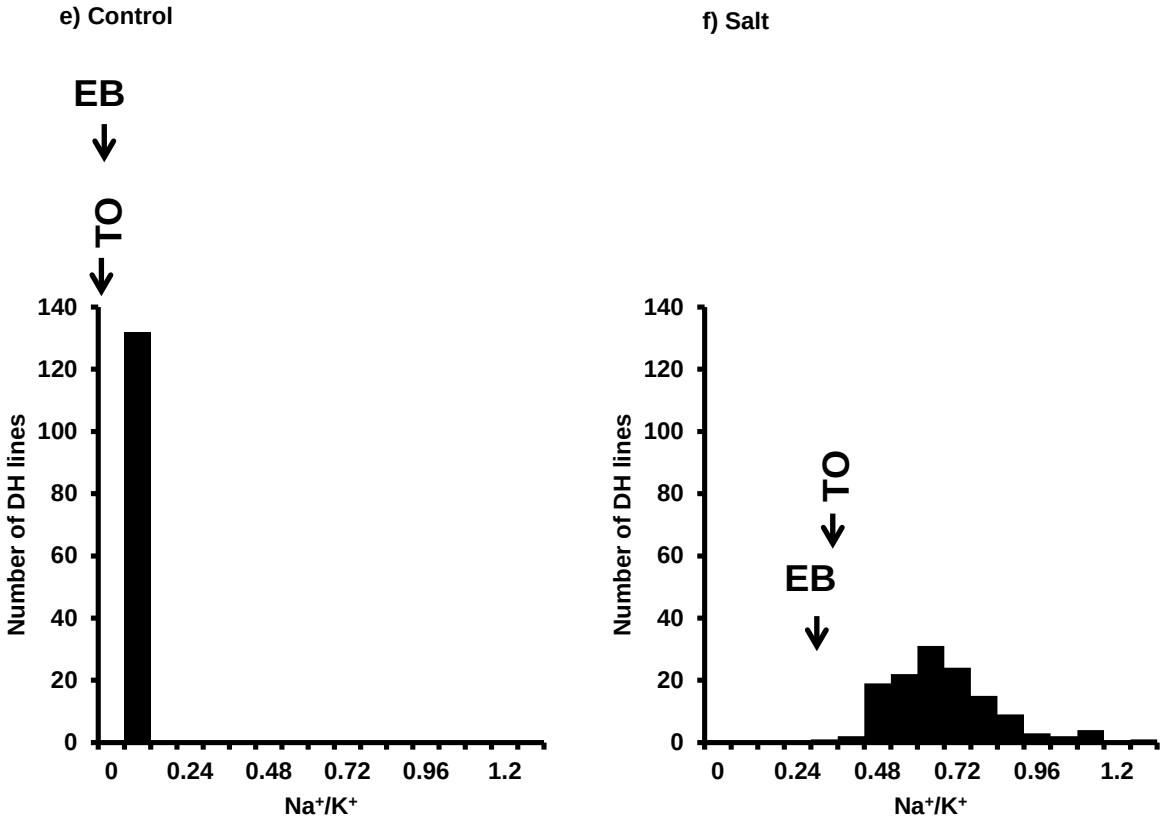


Table V-2: Spearman's rank correlation of growth traits for *Brassica oleracea* Bo1TDH under control conditions

	FW (g)	DW (g)	RWC	SPAD	Na^+ mg/ g DM	K^+ mg/ g DM
Dw (g)	0.85**					
RWC	0.33**	-0.13				
SPAD	0.12	0.20*	-0.11			
Na^+ mg/ g DM	-0.14	-0.30	0.17*	-0.23**		
K^+ mg/ g DM	0.20*	0.01	0.32**	-0.27**	0.23**	
Na^+ /K^+	-0.21*	-0.30**	0.02	-0.11	0.88**	-0.20*

Table V-3: Spearman's rank correlation of growth traits for *Brassica oleracea* Bo1TDH under salt stress (100 mM NaCl) conditions

	FW (g)	DW (g)	RWC	SPAD	Na^+ mg/ g DM	K^+ mg/ g DM
Dw (g)	0.83**					
RWC	0.226**	-0.21*				
SPAD	0.041	0.17*	-0.12			
Na^+ mg/ g DM	-0.161	-0.04**	0.20*	-0.30**		
K^+ mg/ g DM	0.332**	0.20*	0.27**	-0.08	-0.25**	
Na^+ /K^+	-0.053	-0.06	0.03	-0.20	0.87**	-0.63**

5.4.3 QTL analysis and localization

A full map consisting of 279 markers was developed by Iñiguez-Luy et al. (2009), (Appendix 4). A framework with a sub-set of markers was constructed from the full map so as to have one marker per five to ten cM wherever possible. Thirty-one QTL were mapped on all LGs under control and salt stress conditions. Information about the logarithms of odds, intervals and flanking markers of the mapped QTL is summarized in Tables 4 and 5. Figure 5 shows the localization of the QTL mapped under control and salt stress. A positive additive effect means that the Early Big alleles increase the corresponding trait, while a negative additive effect means that the TO1000DH3 alleles increase the corresponding trait

Fresh weight

Four QTL were mapped under control conditions: FW-1C, FW-2C, FW-3C and FW-4C. FW-3C was detected on LG C3 with significance level *P* = 0.05. This QTL alone explains 19% of the phenotypic variation with a negative additive effect. The remaining three QTL together explain 32% of the phenotypic variation and were detected on LGs C1, C3 and C7, respectively. QTL FW-1C has a positive additive effect, while the remaining two QTL have negative additive effects (Table 4). Under salt stress, two QTL, FW-1S and FW-2S, were identified on LGs C1 and C3, respectively. FW-1S explains 6% of the phenotypic variation with a positive additive effect. FW-2S explains 17% of the phenotypic variation and shows a negative additive effect (Table 5).

Dry weight

Under control conditions, one QTL, DW-1C was mapped at *P* = 0.05 on LG C3. This QTL explains 9% of the phenotypic variation. Similarly, under salt stress, one QTL, DW-1S, was mapped on the same LG C3 at *P* = 0.05, which explains 16% of the phenotypic variation. The two QTL, DW-1C and DW-1S show negative additive effects (Tables 4 and 5).

Relative water content

One QTL, RWC-1C, was identified under control conditions on LG C3 at *P* = 0.05, which explains 8% of the phenotypic variation with a negative additive effect (Table 4). No QTL were mapped under salt stress.

Chlorophyll content

Nine QTL were mapped for the SPAD trait. Under control conditions, four QTL SPAD-1C, SPAD-2C, SPAD-3C and SPAD-4C were mapped on the LGs C2, C4 and C8, respectively. SPAD-1C and SPAD-2C were localized on LG C2 at 67 cM and 52 cM, respectively. Collectively, they explain 38% of the phenotypic variation. SPAD-1C, SPAD-2C and SPAD-3C show negative additive effects. SPAD-4C exhibits a positive additive effect (Table 4). Under salt stress, five QTL SPAD-1S, SPAD-2S, SPAD-3S, SPAD-4S and SPAD-5S were mapped. All of them are at *P* = 0.05. They explain 80% of the phenotypic variation. SPAD-1S, SPAD-2S and SPAD-4S were localized on LGs C3, C4, and C6, respectively, with negative additive effects. SPAD-3S and SPAD-5S were mapped on LGs C4 and C8 with positive additive effects (Table 5).

Sodium content

Under the control conditions, one QTL, Na-1C was detected on LG C9, which explains 16% of the phenotypic variation with a positive additive effect. Four QTL were mapped under salt stress, i.e. Na-1S, Na-2S, Na-3S and Na-4S on LGs: C1, C5, C8 and C9, respectively. Overall, they explain 38% of the phenotypic variation. For all of them the additive effects are negative. All of these QTL were mapped at *P* = 0.05 (Tables 4 and 5).

Potassium content

Three QTL, K-1C, K-2C and K-3C, were mapped under control treatment on the respective LGs C1, C3 and C8. They explain 26% of the phenotypic variation. K-2C was mapped at *P* = 0.05 with a negative effect. K-1C and K-3C show positive additive effects (Table 4). Two QTL, K-1S and K-2S, were mapped under salt treatment on LGs C1 and C8, respectively. K-1S and K-2S explain 16% of the phenotypic variation, whereas K-1S showed a negative additive effect. The QTL, K-2S was mapped at *P* = 0.05. This QTL exhibits a positive additive effect (Table 5).

Sodium- potassium ratio

No QTL were found for Na^+/K^+ under control conditions, while three QTL under salt stress were mapped: Na/K-1S, Na/K-2S and Na/K-3S were localized on LGs C1, C8 and C9, respectively. They account for 28% of the phenotypic variation. The QTL Na/K-2S was mapped at *P* = 0.05. The QTL Na^+/K^+-1S and Na^+/K^+-2S show negative additive effects, while Na/K-3S shows a positive additive effect (Table 5).

Table V-4: QTL detected at LOD > 2 under control treatment (C) for fresh weight, dry weight, SPAD1, SPAD2, relative water content, Sodium content Na^+ mg/ g DM and Potassium content K^+ mg/ g DM in *Brassica oleracea* mapping population Bo1TBDH. (QTL significant with *P* = 0.05 are marked bold)

Trait	Name of QTL	LG	LOD	Position (cM)	Interval	Flanking markers	Additive Effect	Phenotypic variation explained (%)
FW	FW-1C	1	3.9	64	60-70	pX101cX -pX122aH	0.24	12.5
FW	FW-2C	3	2.8	31	28-39	fito262 -fito156c	-0.27	9.0
FW	**FW-3C**	**3**	**6.0**	**57**	**51-63**	**pX111aD -fito394**	-0.39	**18.5**
FW	FW-4C	7	3.1	96	91-109	CHS28aX -fito098a	-0.21	10.0
DW	**DW-1C**	**3**	**2.9**	**59**	**52-64**	**pX111aD -fit394**	-0.03	**9.3**
RWC	**RWC-1C**	**3**	**2.5**	**39**	**32-48**	**fito262 -fito156c**	-0.44	**8.1**
SPAD	**SPAD-1C**	**2**	**2.4**	**67**	**64-80**	**fito081a -pW161aX**	-1.07	**7.8**
SPAD	**SPAD-2C**	**4**	**3.7**	**52**	**47-59**	**pW193bE -fito139b**	-1.67	**13.4**
SPAD	SPAD-3C	4	2.5	108	101-116	BRMS034 -pW177bH	-1.14	8.0
SPAD	SPAD-4C	8	3.6	31	25-36	fito482 -pW231aX	1.41	11.4
Na mg/ g DM	**Na-1C**	**9**	**5.1**	**15**	**12-21**	**pW256bH -fito163**	0.20	**16.3**
K mg/ g DM	**K-1C**	**1**	**2.2**	**40**	**37-47**	**pW249dE -fito094**	1.33	**7.4**
K mg/ g DM	**K-2C**	**3**	**3.4**	**65**	**58-70**	**fito394 -fito476**	-1.92	**11.0**
K mg/ g DM	K-3C	8	2.3	69	60-77	pX130cD -fito373c	1.42	7.8

- Linkage group = LG
- Additive effect was calculated by subtracting TO1000DH3 allele by Early Big allele.

Table V-5: QTL detected at LOD > 2 under salt treatment (S) for fresh weight, dry weight, SPAD1, SPAD2, relative water content, Sodium content Na^+ mg/ g DM and Potassium content K mg/ g DM in *Brassica oleracea* mapping population Bo1TBDH. (QTL significant with *P* = 0.05 are marked bold)

Trait	Name of QTL	LG	LOD	Position (cM)	Intervals	Flanking Markers	Additive Effect	Phenotypic variation explained (%)
FW	FW-1S	1	2.0	87	80-91	pW225a -pW239bX	0.13	6.6
FW	FW-2S	3	5.3	52	43-55	pW125dE -pX111aD	-0.25	16.4
DW	**Dw-1S**	**3**	**5.2**	**37**	**31-43**	**fito262 -fito156c**	**-0.04**	**16.0**
SPAD	**SPAD-1S**	**3**	**3.0**	**95**	**91-104**	**BRMS017 -FC**	**-1.30**	**9.8**
SPAD	**SPAD-2S**	**4**	**9.3**	**72**	**66-76**	**pX130aD -pW178bH**	**-1.93**	**26.9**
SPAD	**SPAD-3S**	**5**	**3.4**	**74**	**71-85**	**fito156a -pW164aE**	**1.16**	**10.7**
SPAD	**SPAD-4S**	**6**	**4.0**	**11**	**4-20**	**isgpa -fito067**	**-1.21**	**12.6**
SPAD	**SPAD-5S**	**8**	**6.6**	**51**	**48-56**	**fito204a -pX130cD**	**1.60**	**19.8**
Na mg/ g DM	**Na-1S**	**1**	**3.5**	**32**	**25-36**	**fito355 -pX149fE**	**-1.68**	**11.3**
Na mg/ g DM	**Na-2S**	**5**	**2.9**	**84**	**79-90**	**pW164aE -pW198bH**	**-1.62**	**9.4**
Na mg/ g DM	**Na-3S**	**8**	**1.8**	**82**	**72-84**	**fito204e -fito486**	**-1.19**	**6.0**
Na mg/ g DM	**Na-4S**	**9**	**3.7**	**15**	**14-18**	**pW256bH -fito163**	**1.71**	**11.9**
K mg/ g DM	K-1S	3	3.6	64	57-69	pX111aD -fito394	-2.10	11.6
K mg/ g DM	**K-2S**	**8**	**4.5**	**72**	**60-80**	**fito373c -fito204e**	**2.15**	**14.3**
Na^+/K^+	Na/K-1S	1	2.3	31	25-36	fito355 -pX149fE	-0.04	7.6
Na^+/K^+	**Na/K-2S**	**8**	**2.7**	**78**	**70-84**	**fito373c -fito204e**	**-0.04**	**8.8**
Na^+/K^+	Na/K-3S	9	3.7	1	0-10	FLC1aH -fito204b	0.05	11.9

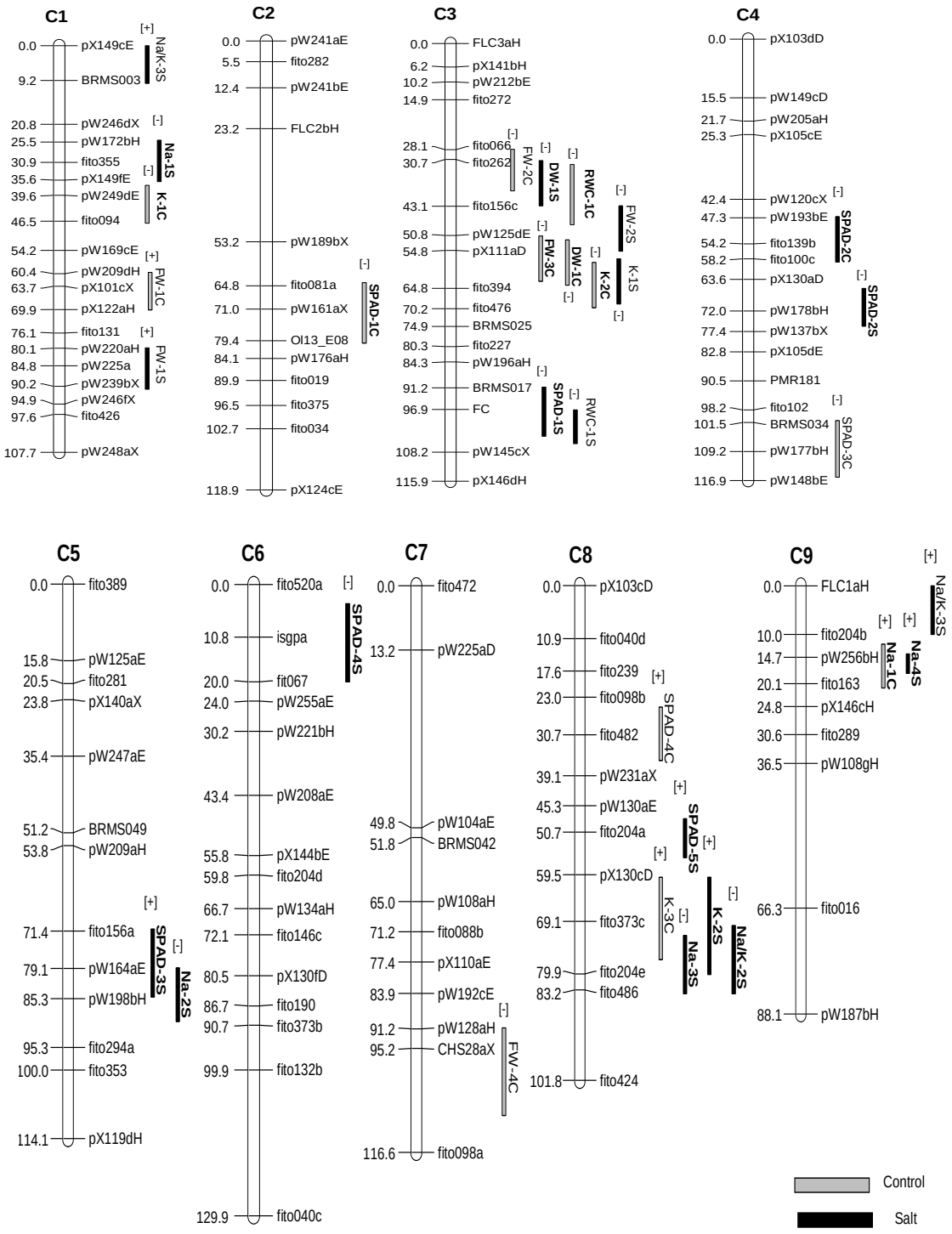


Figure V-5: Localization of QTL for growth traits in *Brassica oleracea* Bo1TBDH population. (QTL significant with *P* = 0.05 are marked bold)

5.5 Discussion I

5.5.1 Traits variations

The reduction in FW and DW was 32% and 17%, respectively. The reduction in FW and DW is mainly due to the deleterious effect of osmotic stress and ion-toxicity. The correlation of Na^+ with FW and DW is negative and significant, which explains the significant reduction in FW and DW. The reduction was 3% in the case of RWC, which means that plants could maintain turgidity even under intensive salt stress by accumulating inorganic solutes such as Na^+ and K^+. This is supported by the positive and significant correlation between RWC and Na^+ and K^+ upon control and salt stress.

Physiologically, the reason for this dramatic decrease is probably engendered by osmotic stress, which causes an immediate stomatal closure. Consequently, there is a reduction in the CO2 concentration, which is an essential compound for photosynthesis (Munns and Tester 2008). Osmotic stress is a tentative event plants can recover from within few hours. For example, in barely under salt stress, 150 mM NaCl; plants took one hour to generate osmotic adjustment (Munns 2002). However, with time, the Na^+ level increases to toxic levels. The overloading of ions, especially, Na^+ and Cl^- is catastrophic either in the cell wall or inside the cell, causing cell dehydration (Munns 2002). When the rate of Na^+ and Cl^- sequestering becomes lower than the rate of influx of Na^+ and Cl^-, the accumulation will be higher in cytoplasm because the size of cytoplasm is small compared with the vacuole size (Munns 2002). This high rate of Na^+ and Cl^- accumulation in cytoplasm is toxic for several enzymes, in particular, those that are K-dependent. Under these conditions, Na^+ acts as a competitive inhibitor for K^+ on the active sites of these enzymes (Munns 2002; Shabala and Cuin 2007).

In our experiment, chlorophyll content measured by SPAD increased by 6%. This increase in SPAD values is mainly due to a reduction of leaf area, which in turn increases the number of chloroplasts per unit area (Fricke et al. 2004). The correlation of SPAD with FW and DW was positive and significant for both control and salt stress conditions. As it was expected, the correlation between SPAD and the traits related to Na^+ was negative and significant, which reflects the negative effect of the high levels of Na^+ in chloroplasts on photosynthesis.

Under salt stress, a dramatic increase of about 130% in Na^+ was observed. The correlation of Na^+ with FW and DW was negative and significant, which explains the significant reduction in FW, DW and K^+ traits. This reduction in plant growth may be due to the replacement of K^+ ions by Na^+ ions, which break down the activities of the K-dependent enzymes (Munns and Tester 2008). Noteworthy is that K^+ negatively and significantly correlated with SPAD under control conditions, probably because the concentration of K^+ exceeded the optimal limits (Subbarao et al. 2003). These authors reported that the optimal concentration for enzymes' maximum activity varies from 10-50 mM. Another possibility for this reduction is the accumulation of Na^+ and Cl^+ in the chloroplasts, which hinders photosynthesis. Contrarily, the correlation of K^+ was positive and significant with FW, DW and RWC, particularly under salt stress. This

correlation means that K^+ is involved in several metabolic pathways such as the activation of various enzymes and K^+-non-specific action as an osmoticum.

The physiological effects of salinity on plant growth are intensively discussed in Chapter 4 (4.5).

5.5.2 QTL analysis and localization

Fourteen QTL were identified under control growth conditions. Several QTL hotspots for different traits were detected on different LGs such as LGs C3, C8 and C9. The major hotspot was observed on LG C3. This hotspot comprises ten QTL for different traits. There are three points of overlapping QTL intervals (Figure 5). There is a major cluster in the middle of LG C3 comprised of the QTL FW-3C, FW-2S, DW-1C, K-2C and K-1S. The cluster of the first three QTL is expected because they are developmentally related traits, as is demonstrated by their positive and significant correlation under both treatments. Similarly, the overlap of the last two QTL is highly expected because they are for the same trait. The interference of the QTL intervals for the morphological traits such as FW and DW with QTL for physiological traits like K^+ indicates that K^+ contributes positively to plant growth. This is supported by the positive and significant correlation between FW, DW and K^+, especially under salt stress. The clustering of these traits can be explained in two possible ways: a pleiotropic effect of one gene that controls the variation of these traits or the effects of tightly linked genes, regulating these traits independently. The second overlapping point was observed at the bottom of this LG, between SPAD-1S and RWC-1S; however, they are negatively and significantly correlated. Probably one gene with pleiotropic effect resides in this genomic region. This gene may increase one trait and decrease the second. Another possibility is the presence of two genes that control the variation of the two traits independently. The third site of overlapping QTL intervals was observed at the top of the LG, which includes the QTL FW-1C, DW-1S and RWC-1C. Based on their positive and significant correlation, the clustering of these QTL is expected. Since, three QTL subgroups were identified on this LG; at least three major genes that govern the variation of these traits are hidden in these genomic regions.

Since the QTL FW-1C and RWC-1C were mapped under control conditions, while DW-1S was mapped under salt stress, the genome harbors gene(s) that control(s) the variation of more than one trait under different growth conditions. Additionally, there are genomic regions where gene(s) that exclusively control(s) the variation of one trait under control and salt, such as K-2C and K-1S. They are stress-non-specific regions or constitutive QTL. Other genomic regions harbor genes that control the variation of different traits under salt stress, like SPAD-1S and RWC-1S. On the other hand, QTL that exclusively regulate the variation of one trait or more under salt stress are stress-specific or adaptive QTL.

Remarkably, the additive effects of all QTL mapped on LG C3 were negative, indicating that the alleles for increasing these traits were transmitted by the same parent; in this case it was TO1000DH3.

Another QTL hotspot was found on LG C8 where six QTL were located. Out of these four QTL are associated with Na^+ and K^+-related traits. K-3C and K-2S show positive additive effects, while the additive effects of Na/K-2S and Na-3S are negative. The opposite additive effects of these QTL suggest that the alleles that control these trait variations are in a repulsion phase. The co-localization of K-2S, Na/K-2S is of great importance. They show a significant and negative correlation under salt stress, which means that K^+ and Na^+/K^+ are inversely proportionate. Thus the flanking markers that are associated with these QTL can be harnessed for selection to increase K content and to decrease Na^+/K^+.

Another QTL hotspot was mapped on LG C9 for Na^+- and K^+-related traits. Three QTL were mapped on the top part of LG C9. Na-1C and Na-4S share the same interval. The third QTL, Na/K-3S, is salt-specific and regulates the variation of Na^+/K^+ under salt stress. This genomic region probably harbors plasma membrane antiporters or vacuolar antiporters. The former governs Na^+ exclusion from the cell into the soil solution, the later manages the influx of excessive Na^+ into the vacuole to be sequestered. Most likely, this QTL is a major gene that controls the Na/K ratio, since this QTL explains 12% of the phenotypic variation. Similarly, QTL for Na^+ and K^+ were mapped for Na^+- and K^+-related traits on LG C9 in the *B. napus* mapping population of Mansholts and Samourai as discussed in Chapter 4 (4.5).

The smallest QTL hotspot was observed on LG C5, where the SPAD-3S and Na-2S QTL overlapped; they are significantly and negatively correlated. This co-localization is of high value because a selection for improving SPAD will be associated with a reduction in Na^+ content.

Conclusion

In conclusion, wide variations were observed in the DH population in all traits. The FW and DW deceased considerably under salt stress. This reduction is imposed by NaCl in two phases, osmotic stress and ion toxicity. The QTL analysis spotted several genomic regions of high interest. In this context, two types of QTL were identified, the stress-specific (adaptive QTL), which explain the variance of one trait or more under either control or salt, and the stress-non-specific (constitutive QTL), were identified. These QTL control the variations in single traits or more under control and salt stress conditions. Of high importance are the QTL that underlie the variations of more than one trait, in particular when the correlation between them is negative. Thus, selection for increasing a desired trait can be achieved along with reducing an undesired trait.

Part II

Mapping QTL for leaf glucosinolates variation under control and salt stress conditions in *B. oleracea*

The experimental design and protocol of GSL analysis are described in detail in Chapter 4 (4.3) and growth conditions earlier in Chapter 5 (5.3.2).

The systematic and common names, the abbreviations and the precursor amino acids of each GSL constituent are listed in Chapter 4 (Table 1). The parents and GSL profiles of the DH populations will be described separately for the control and salt treatment.

5.6 Results II

The parents and the DH population were tested for GSL variation under control conditions and under salt stress with 100 mM NaCl, in order to elucidate the effect of salinity under both growth conditions. The parents of population TO1000DH3 and Early Big showed broad GSL variation. TO1000DH3 had high GSL content compared to Early Big.

5.6.1 Parents' GSL profile and content under control conditions

There was a considerable difference in GSL content between TO1000DH3 and Early Big. The total GSL concentrations of the parental lines were 11.3 µmol/ g DM for TO1000DH3 and 4.67 µmol/ g for Early Big. Unfortunately, as a result of poor germination of Early Big under salt stress, we could not determine its GSL content, but we could measure a total GSL content of 6.7 µmol/ g DM for TO1000DH3 (Table 6, Figure 6a).

TO1000DH3 GSL content under control conditions

Under control conditions, TO1000DH3 exhibited a total GSL content of 11.3 µmol/ g DM. The major GSL constituents belong to the aliphatic class with 8.9 µmol/ g DM, which represent 79% of the total GSL (Table 8). Four aliphatic GSL components were identified: GNA GNL, IBE and PRO. GNA dominated the other individuals with of 5.7 µmol/ g DM with contributions of 63% and 50% in the indolic and the total GSL, respectively. The remaining components, GNL, IBE and PRO, were detected in concentrations of 1.8 µmol/ g DM, 0.94 µmol/ g DM and 0.5 µmol/ g DM, respectively, representing a respective 20%, 10% and 6% of the aliphatic GSL. The contributions of these components to the total GSL content are 16%, 8% and 4%, respectively (Table 6, Figure 6a, b).

Indolic GSL represent the smallest class. It is much lower than the aliphatic class, with 10% of the total GSL and a concentration of 1.2 µmol/ g DM (Figures 6a, b and 7a, b and Table 7). Three constituents were detected: GBC is the dominant component with 1.1 µmol/ g DM, representing 89% of the indolic GSL and 9% of the total GSL content. Concentrations of the remaining components 4OH and NAS are

rather low with 0.015 µmol/ g DM and 0.11 µmol/ g DM, respectively. The contributions of both components are 11% and 1% in the indolic GSL and the total GSL (Figures 6b and 7b and table 7).

The aromatic GSL is represented by a single component, NEO, with a concentration of 1.3 µmol/ g DM, equaling 11% of the total GSL (Figures 6b and 9 and Tables 6, Table 7).

Early Big GSL content under control conditions

Unexpectedly, under control conditions, a single aliphatic component, GNL, was detected. The GNL concentration is 1 µmol/ g DM. The ratio of aliphatic GSL to total GSL is 22% of the total GSL (Figure 6b and Table 7).

The indolic GSL are the most abundant, with 3.13 µmol/ g DM. This amount accounts for 67% of the total GSL. Only two constituents were detected, GBC and 4OH, in concentrations of 3.1 µmol/ DM and 0.03 µmol/ g DM, respectively. Their respective shares in the total GSL are 66% and less than 1% (Figure 6a, b and Table 7).

In the case of aromatic GSL, one component (NEO) was measured in a concentration of 0.5 µmol/ g DM, which represents 11% of the total GSL amount (Figure 9 and Table 7).

Overall, TO1000DH3 shows higher GSL content than Early Big, with ratios of 240%, 870%, 37% and 252%, for total GSL content, aliphatic GSL, indolic and aromatic GSL, respectively. These findings reveal that Early Big dominates TO1000DH3 only in indolic GSL content.

5.6.2 Parents' GSL profile and content under salts tress conditions

TO1000DH3 GSL content under salt stress

Under salt stress, total GSL in TO1000DH3 declined considerably, by 41% relative to the concentration under control, being 6.8 µmol/ g DM (Figure 6a and Table 7). The aliphatic GSL concentration was 3.96 with 59% of the total GSL. A new GSL component was estimated under salt stress GBN in a low concentration of 0.03 µmol/ g DM, which represents less than 1% of the aliphatic GSL. Reductions in single individuals were significant. PRO disappeared completely. The major ingredients GNA and IBE were found in concentrations of 3.15 µmol/ g DM and 0.72 µmol/ g DM. Their shares in the aliphatic GSL were 79% and 18%, respectively. They contributed a respective 47% and 11% to the total GSL (Figure 6a and Table 6). The minor constituents GNL and GBN were found in rather low concentrations of 3% in the aliphatic GSL and less than 1% of the total GSL (Figure 6b and Table 7).

In contrast, indolic GSL increased from 1.2 µmol/ g DM under control conditions to 1.5 µmol/ g DM, an increase of 22%. They represent 22% of the total GSL content (Figure 6a, b and Table 7). Three components were identified: GBC, NAS and 4OH. The

absolute concentrations of these ingredients are 1.2 µmol/ g DM, 0.34 µmol/ g DM, respectively. 4OH was estimated in a much lower concentration of 0.03 µmol/ g DM (Figure 7a and Table 6). The contributions of these components to the indolic GSL are 76 %, 22% and 2%, respectively (Figure 7b). Their percentages of the total GSL are 17% for GBC and 5% for NAS and less than 1% for 4OH (Figure 6b).

The single aromatic GSL component, NEO, slightly increased by 2% being 1.3 µmol/ g DM, which represented 19% of the total GSL (Figure 9 and Table 7)

Early Big GSL content under salt stress

Regrettably, we cannot report on the GSL profile under salt stress of the second parent, Early Big, due to the lack of analyzable plant material as a consequence of poor germination. Therefore, we could not count how many DH lines showed GSL content lower than Early Big under salt stress.

5.6.3 Glucosinolates' profile and content of the DH population under control conditions

In the DH population, a significant difference in the glucosinolate profile and content was observed under control and salt stress conditions. The total glucosinolate content varied from 2.3 µmol/ g DM to 15.7 µmol/ g DM with an average of 8.5 µmol/ g DM under control. Under the salt regimen, total GSL concentrations varied from 0.92 µmol/ g DM to 10.8 µmol/ g DM, with a mean value of 4.4 µmol/ g DM (Tables 5 and 6). The distribution of the total GSL content under control and salt stress conditions showed a transgressive segregation (Figure 10). Mostly, the single constituents showed normal distributions (Appendix 13). Under salt stress, no lines with a lower GSL content than Early Big, the parent with low GSL content, could be identified (Figure 10). A significant genotypic variation was observed among the DH lines; the genotypic variation and heritabilities are outlined in Appendix 12.

Aliphatic GSL under control conditions

With a contribution of 37% to the total GSL, the aliphatic GSL class is not the largest. The mean value of the aliphatic GSL is 2.6 µmol/ g DM ranges between 0 µmol/ g DM and 10.8 µmol/ g DM (Table 7). The main components, GNA, PRO, IBE and GNL, were detected in concentrations of 1.2 µmol/ g DM, 0.58 µmol/ g DM, 0.57 µmol/ g DM and 0.26 µmol, respectively. The proportions of these components in the aliphatic GSL are 46%, 22%, 22% and 10%, respectively. Their contributions to the total GSL are 13%, 8%, 8% and 4%, respectively (Table 6, Figures 6a, b). RAA was much lower than the remaining components, with 0.02 µmol/ g DM (Figure 6a, b Table 6).

Indolic GSL under control conditions

Indolic GSL class represents the highest portion of total GSL, with 40%. The mean value is 2.8 µmol/ g DM (Table 7). The DH line values range from 0.32 µmol/ g DM to 9.26 µmol/ g DM. The superabundant ingredient is GBC, with 2.5 µmol/ g DM. The percentages of GBC are 91% of indolic GSL and 36% of the total GSL. The remaining components, NAS, 4ME and 4OH, show mean values as high as 0.13 µmol/ g DM,

0.11 µmol/ g DM and 0.03 respectively. The contributions of these components together are 9% indolic GSL and 5% total GSL.

Aromatic GSL under control conditions

As usual, the aromatic GSL class is represented by one constituent, NEO, with a value of 1.6 µmol/ g DM, equaling 23% of the total GSL content. The values of NEO range from 0.25 µmol/ g DM to 6.7 µmol/ g DM (Figure 9 and Table 7).

5.6.4 Glucosinolates' profile and content of the DH population under salt stress conditions

Total GSL concentration and composition were found to be modified under salt stress with 100 mM NaCl. Under salt stress, the total GSL content significantly declined by 49%. Total GSL concentrations varied from 0.92 µmol/ g DM to 10.8 µmol/ g DM, with a mean value of 4.4 µmol/ g DM (Table 6).

Aliphatic GSL under salt stress conditions

Aliphatic GSL fell by 32% to 1.8 µmol/ g DM, which represents 42% of total GSL content (Table 6). The aliphatic GSL measurements range from 0 µmol/ g DM to 10.8 µmol/ g DM. The concentrations of the major components, GNA, IBE and PRO, are 0.93 µmol/ g DM, 0.38 µmol/ g DM, and 0.33 µmol/ g DM, respectively. The shares of these components in the aliphatic GSL are 53%, 22% and 18%, and their constituents represent 23%, 9% and 8% of the total GSL, respectively. The minor components, with much lower concentrations, are GNL, RAA and GBN. Collectively, they share 7% of the aliphatic GSL and 3% of the total GSL content (Table 6 and Figure 6b, and 7b).

Indolic GSL under salt stress conditions

The total concentration of indolic GSL fell significantly by 48% to 1.5 µmol/ g DM, is 35% of the total GSL content (Table 7). The concentration of indolic GSL ranges from 0.24 µmol/ g DM to 3.8 µmol/ g DM (Table 6). Four constituents were quantified. The effect of salinity on the analyzed species differed: while two, GBC and 4ME, decreased, NAS showed no change and 4OH increased. The two major components, GBC and NAS, were detected in values of 1.2 µmol/ g DM, 0.13 µmol/ g DM, respectively. Their contributions to the indolic GSL were 86% and 9%, and their shares in the total GSL were 30% and 3%, respectively. The two minor components, 4ME and 4OH, were detected in much lower concentrations with less than 6% of the indolic GSL and less than 3% of the total GSL (Figure 8a, b, and Table 6).

Aromatic GSL under salt stress conditions

The aromatic GSL are represented by NEO, a single component with a value of 0.87 µmol/ g DM, i.e. 22% of the total GSL. The drop of 47% in absolute concentration is dramatic. The values of NEO range from 0.19 µmol/ g to 4.04 µmol/ g DM (Figure 9 and Table 7).

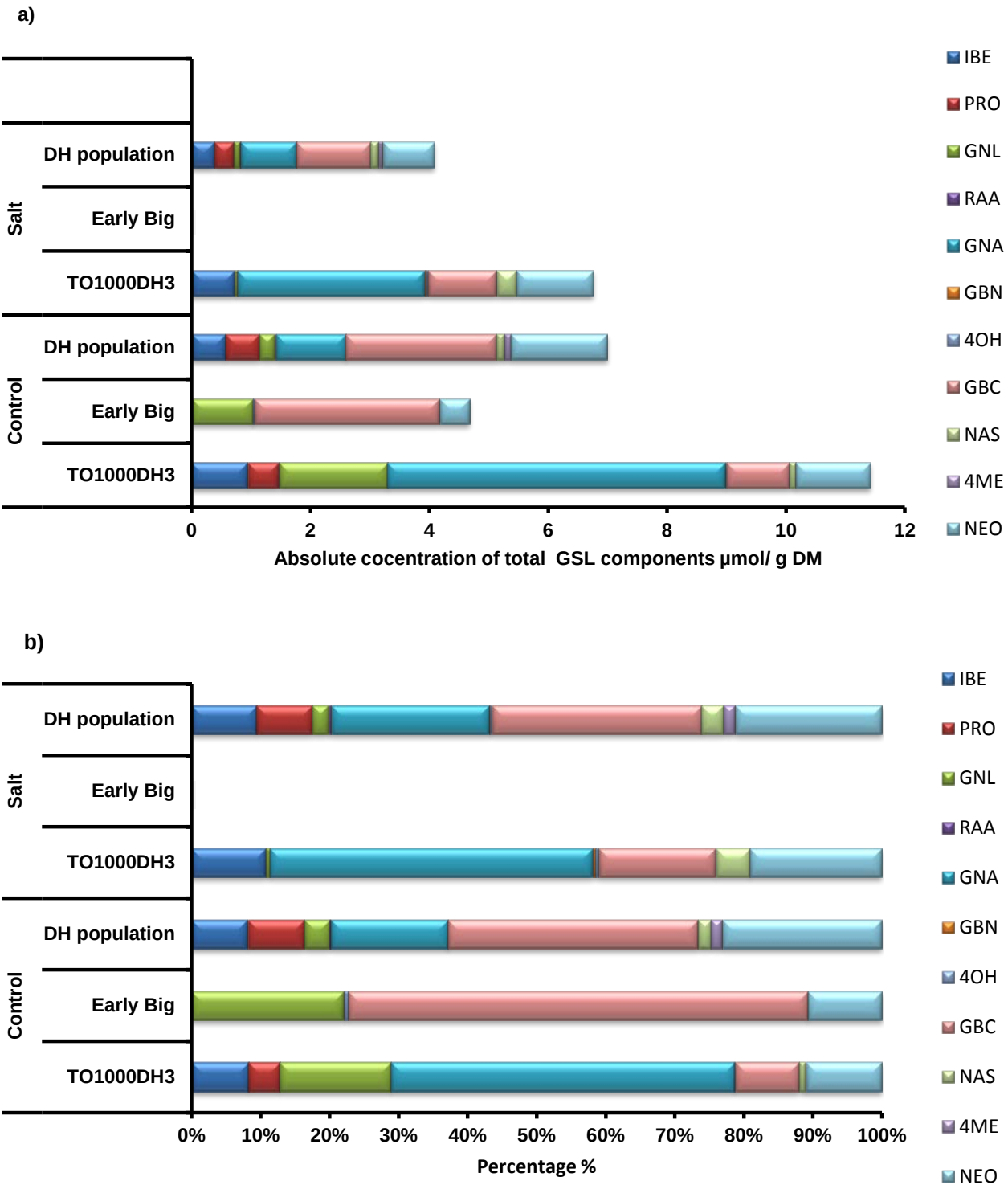


Figure V-6: (a) Total glucosinolate content µmol/ g DM of each component in parents and the DH population under control and salt, (b) Percentage of each component relative to the total GSL content in parents and the DH population under control and salt

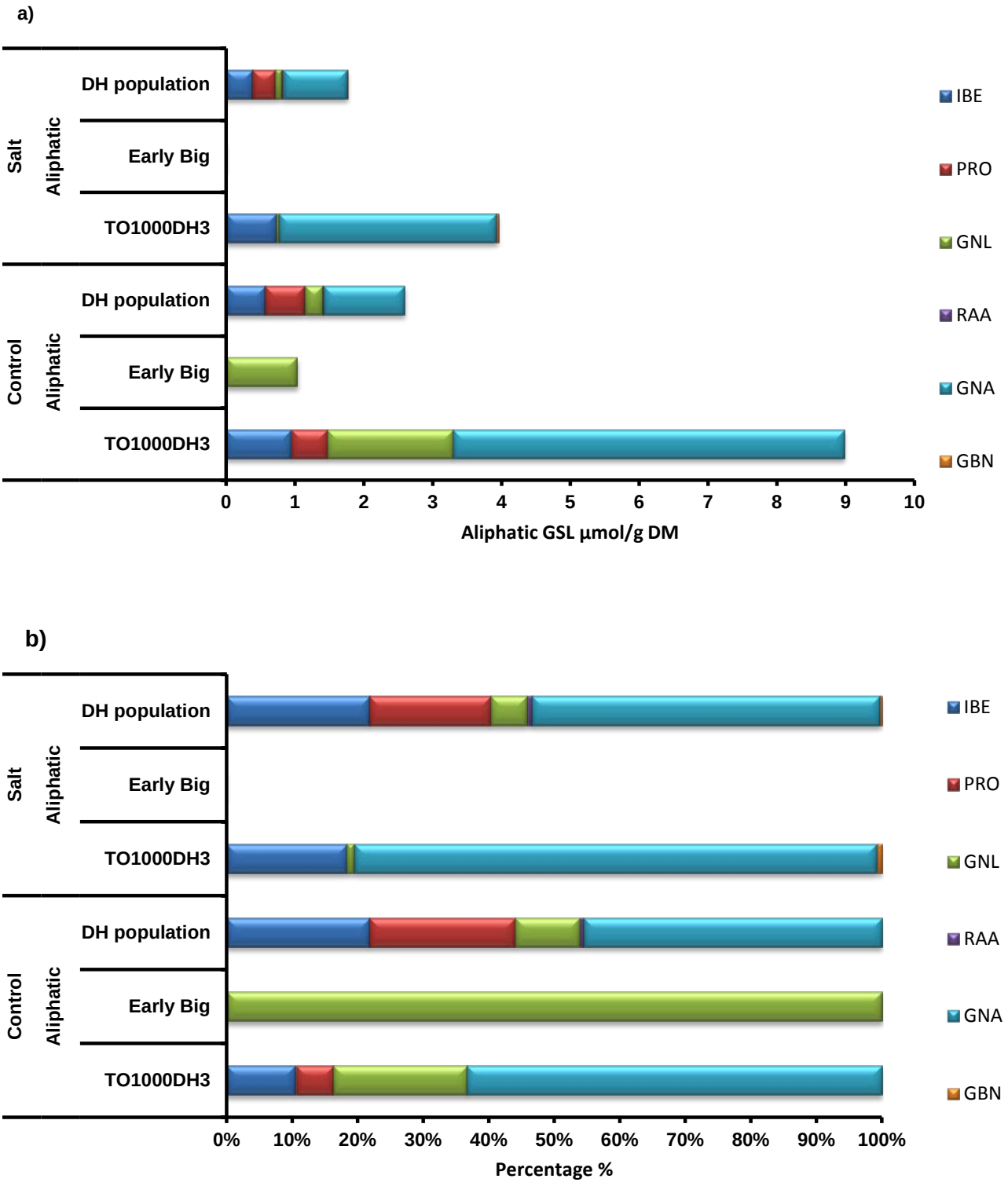


Figure V-7: Absolute values of aliphatic GSL components µmol/ g DM in the parents and the DH population under control and salt, b) Percentage of each ingredient of the aliphatic GSL in the aliphatic GSL content in parents and the DH population under control and salt

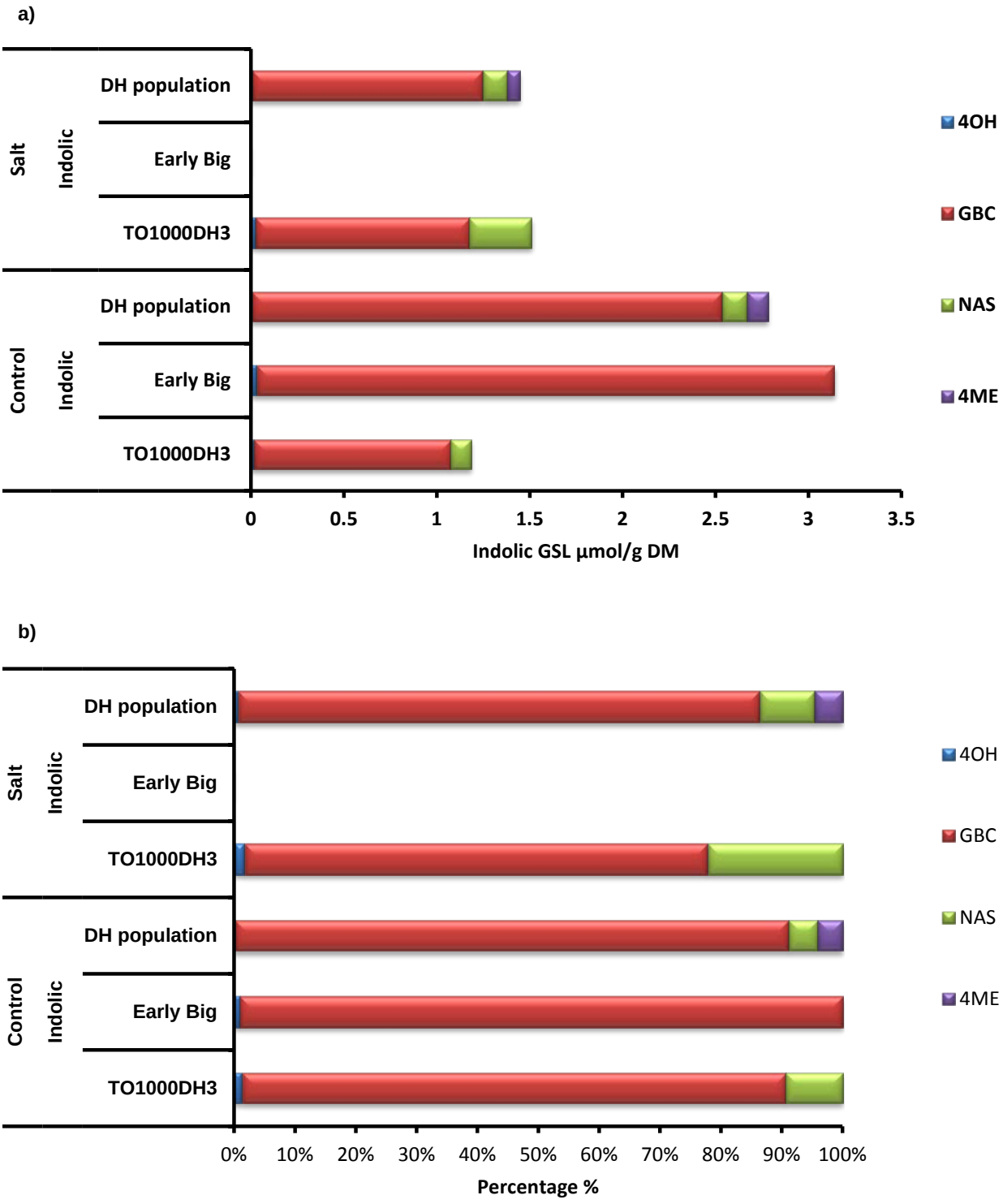


Figure V-8: Absolute values of indolic GSL components µmol/ g DM in the parents and the DH population under control and salt, b) Percentage of each ingredient of indolic GSL in the total indolic GSL content in parents and the DH population under control and salt

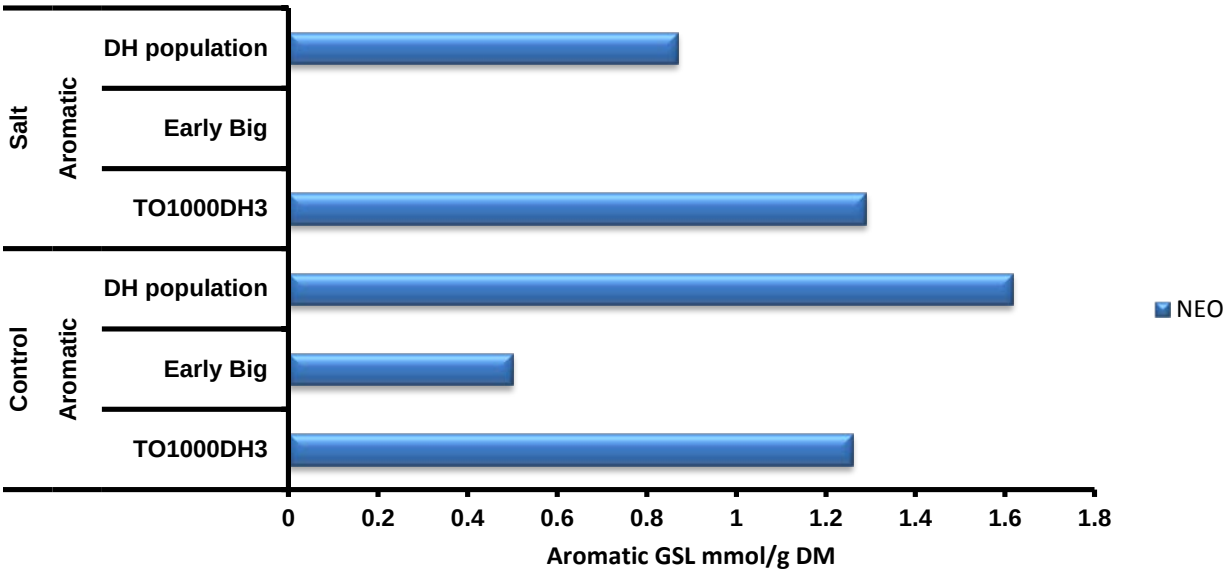


Figure V-9: The aromatic GSL content µmol/ g DM in parents and the DH population under control and salt

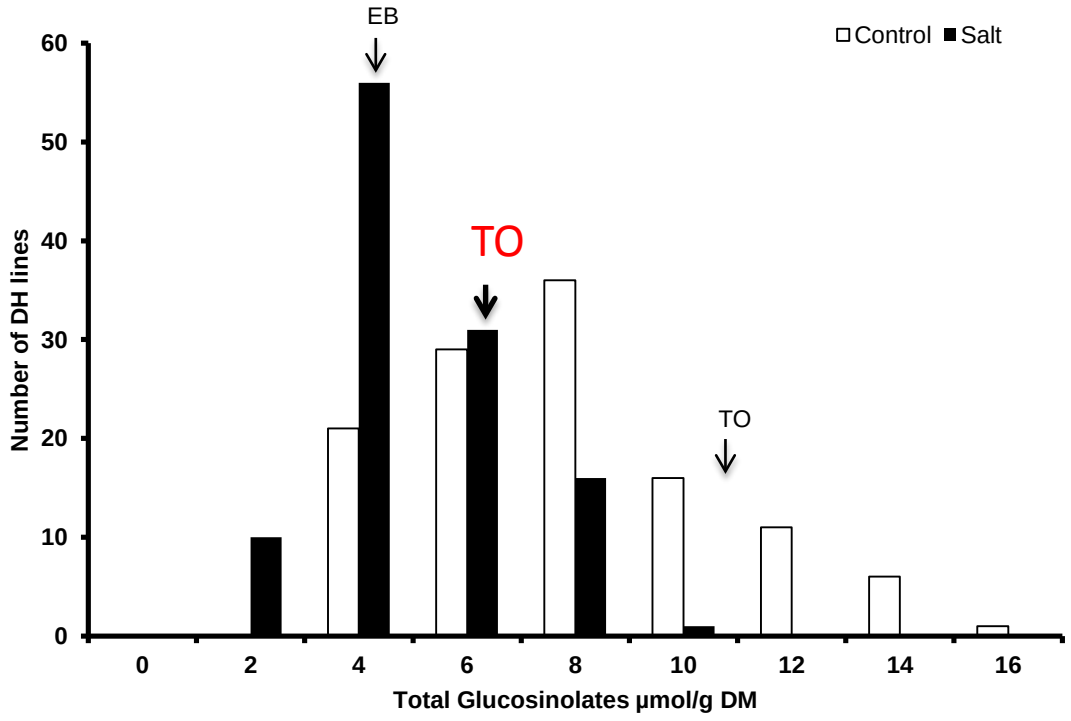


Figure V-10: Distribution of total GSL µmol/ g DM of *Brassica oleracea* DH population under control and salt stress

Table V-6: Minimum, maximum and mean of glucosinolate content µMol/ g DM of *Brassica oleracea* mapping population Bo1TBDH and parents under control and salt treatment (100 mM NaCl)

	Minimum		Maximum		Mean		TO1000DH3		Early big	
Traits	control	salt	control	salt	control	salt	Control	salt	control	salt
IBE	0.00	0.00	2.74	1.51	0.57	0.38	0.94	0.72	0.00	-
PRO	0.00	0.00	6.39	2.45	0.58	0.33	0.53	0.00	0.00	-
GNL	0.00	0.00	3.25	1.89	0.26	0.01	1.83	0.045	1.03	-
RAA	0.00	0.00	0.22	0.16	0.02	0.01	0.00	0.00	0.00	-
GNA	0.00	0.00	9.17	5.44	1.18	0.93	5.69	3.15	0.00	-
4OH	0.00	0.00	0.11	0.15	0.003	0.01	0.02	0.03	0.03	-
GBN	0.00	0.00	0.00	0.09	0.00	0.01	0.00	0.03	0.00	-
GBC	0.15	0.14	8.30	3.49	2.53	1.24	1.10	1.15	3.11	-
NAS	0.00	0.00	0.86	0.67	0.13	0.13	0.11	0.33	0.00	-
4ME	0.00	0.00	0.65	0.32	0.11	0.08	0.00	0.00	0.00	-
NEO	0.04	0.19	6.66	4.03	1.62	0.90	1.26	1.30	0.50	-
SUM	2.30	0.92	15.70	10.19	8.50	4.41	11.42	6.80	4.67	-

Table V-7: Minimum, maximum and mean values of the major categories; aliphatic, indolic and aromatic GSL µmol/ g DM of *Brassica oleracea* mapping population Bo1TBDH under control condition and salt treatment (100 mM NaCl)

Traits	DH population								TO1000DH3				Early Big			
	Minimum		Maximum		Mean		Percentage		Mean		Percentage		Mean		Percentage	
	C	S	C	S	C	S	C	S	C	S	C	S	C	S	C	S
Aliphatic	0.00	0.00	10.76	6.03	2.59	1.77	37%	43%	8.98	3.96	79%	59%	1.03	-	22%	-
Indolic	0.32	0.22	9.26	3.76	2.78	1.45	40%	35%	1.19	1.51	10%	22%	3.14	-	67%	-
Aromatic	0.25	0.19	6.66	4.04	1.62	0.87	23%	22%	1.26	1.29	11%	19%	0.50	-	11%	-

- C: Control S: Salt

5.6.5 Correlations

Under control conditions, the correlations between the individuals belonging to the aliphatic class are positive, except the correlation between IBE and GNA, which is negative and significant (r = -0.25**) and PRO (r = -0.24**), (Table 8). In the case of indolic GSL, the correlations between all components are positive, except between GBC and 4OH, where it is negative and significant (r = -0.22*). Mostly, the correlation is negative between aliphatic GSL components with the indolic GSL components (Table 8). The aromatic GSL single component shows positive correlations with all aliphatic and indolic types; the only exceptions is with IBE (r = -0.19*).

Under salt stress, the correlations are positive among the different aliphatic GSL components (Table 9). Likewise, the indolic GSL constituents reveal positive correlations, except between NAS and 4ME, where the correlation is negative and significant (r = -0.62**). The aliphatic components correlate positively and significantly with the indolic ones, except between NAS and PRO, where it is negative and significant correlation (r = 0.20*). The aromatic GSL ingredient NEO correlates positively with all indolic and aliphatic components, except with GNL and GNA, where it is negative and non-significant (Table 9).

Table V-8: Spearman's rank correlations of glucosinolates in *Brassica oleracea* mapping population Bo1TBDH under control condition.

	IBE	PRO	GNL	RAA	GNA	4OH	GBC	NAS	4ME	NEO
PRO	-0.24**									
GNL	0.01	0.14								
RAA	-0.03	-0.07	0.13							
GNA	-0.25**	0.61**	0.30**	0.07						
4OH	0.01	0.07	0.10	0.43**	0.04					
GBC	0.06	-0.16	-0.02	0.061	-0.11	-0.22*				
NAS	-0.03	-0.14	0.06	0.20*	0.06	0.15	0.06			
4ME	0.07	0.03	0.01	0.16	-0.01	-0.07	0.18*	-0.31		
NEO	-0.18*	0.09	0.04	0.09	0.04	0.001	0.092	0.05	-0.004	
SUM	0.01	0.38**	0.24**	0.10	0.49**	0.13	0.52**	0.10	0.10	0.42**

Table V-9: Spearman's rank correlations of glucosinolates in *Brassica oleracea* mapping population Bo1TBDH under salt stress (100 mM NaCl)

	IBE	PRO	GNL	RAA	GNA	GBN	4OH	GBC	NAS	4ME	NEO
PRO	0.15										
GNL	0.05	0.07									
RAA	0.001	-0.16	0.05								
GNA	0.04	0.11	0.08	0.03							
GBN	0.05	0.22*	0.06	0.29**	-0.02						
4OH	0.20	0.12	0.11	0.27**	0.16	0.54**					
GBC	0.16	-0.05	-0.01	0.05	0.02	0.16	0.18				
NAS	-0.13	-0.20*	0.07	0.19*	-0.02	0.03	0.03	-0.02			
4ME	0.15	0.23*	0.04	-0.02	0.07	0.10	0.18*	0.31**	-0.62**		
NEO	0.02	0.01	-0.05	0.20	-0.09	0.26**	0.04	0.15	0.14	0.005	
SUM	0.24**	0.31**	0.14	0.02	0.56**	0.19*	0.22*	0.48**	0.03	0.22*	0.31**

5.6.6 QTL analysis and localization under control and salt stress conditions

Thirty-two QTL were mapped. Under control conditions, 21 QTL were identified and 11 QTL under salt. A full description of the QTL, flanking marker, additive effects, and the percentage of variance explained by each QTL and their localization on LGs is giving in Tables 10 and 11 and in Figure 11). If the QTL show negative additive affects, the alleles for increasing the corresponding trait were inherited from TO100DH3. QTL with positive additive effects indicate that the alleles for increasing the trait were passed on by Early Big.

QTL associated with the aliphatic GSL under control and salt stress conditions

With regard to IBE under control conditions, two QTL, IBE-1C and IBE-2C, were mapped on LGs C1 and C5, respectively. IBE-1C and IBE-2 account for 23% of the phenotypic variation, while IBE-1C was detected at the significance level $P = 0.05$. The additive effects of both QTL are negative. Under salt stress one QTL, IBE-1S, was identified on LG C2 with a positive additive effect. The percentage of variation explained by this QTL is 5%. One QTL, PRO-1C, was found positioned on LG C3 under control conditions. PRO-1C was mapped at a significance level of $P = 0.05$, with a positive additive effect and the percentage of phenotypic variation explained by this QTL is 16%. Under salt stress, two QTL were localized: PRO-1S on LG C3 and PRO-2S on C8, both QTL show negative additive effects. These two QTL explain 26% of the phenotypic variation. No QTL were mapped under control conditions for RAA. Under salt stress, the QTL RAA-1S was localized on LG C9. This QTL was mapped at $P = 0.05$, shows a positive additive effect, and explains 7% of the phenotypic variation. One QTL for GNL, GNL-1C, was identified on LG C5. This QTL shows a negative additive effect and explains 6% of the phenotypic variation. No QTL were mapped for GNL under salt stress. Under the control conditions, three QTL, GNA-1C, GNA-2C and GNA-3C were localized on LGs C3, C7 and C9, respectively. They explain 54% of the observed phenotypic variation, where GNA-3C alone accounts for 33%. GNA-2C and GNA-3C were detected at the significance level $P = 0.05$. The additive effects for all of them were negative. Under salt stress, one QTL, GNA-1S, was identified on LG C7, which accounts for 15% of the phenotypic variation, and shows a negative additive effect. For total aliphatic GSL content two QTL, Alipahatic-1C and Aliphatic-2C, were identified on LGs C7 and C9, respectively. Aliphatic-1C and Aliphatic-2C were identified at $P = 0.05$. Both QTL show negative additive effects and they account for 50% of the observed phenotypic variation. Under salt stress, one QTL, Aliphatic-1S, was detected on LG C7 with a positive additive effect. This QTL was identified at $P = 0.05$. The fraction of variation explained by this QTL is 15%.

QTL associated with the indolic GSL under control and salt stress conditions

In the case of GBC, three QTL, GBC-1C, GBC-2C and GBC-3C were detected on LGs C2, C3 and C9, respectively. All of them exhibit positive additive effects. GBC-1C and GBC-3C were mapped at $P = 0.05$. All of them together explain 28% of the phenotypic variation. One QTL, GBC-1S, was identified under salt stress on LG C9. This QTL accounts for a 17% of the phenotypic variation. This QTL was mapped at $P = 0.05$. Under control conditions, one QTL, NAS-1C, was detected on LG C4. This QTL explains 8% of the phenotypic variation and shows a negative additive effect. Similarly, one QTL, NAS-1S, was found under salt stress on LG C4 and indicates a positive additive effect. The percentage of variation explained by this QTL is 6%. No QTL were identified for 4ME under control, but one QTL, 4ME-1S, was

localized under salt stress on LG C5. This QTL explains 5% of the observed variation and shows a positive additive effect. For total indolic GSL under control conditions, three QTL, Indolic-1C, Indolic-2C and Indolic-3C, were mapped on LGs C2, C3 and C9, respectively. The additive effects are positive for all QTL. Collectively, they explain 26% of the observed phenotypic variation. Indolic-2C was mapped at *P* = 0.05. Under salt stress, one QTL, Indolic-1S, was found at *P* = 0.05 on LG C9. This QTL explains 17% of the phenotypic variation of the indolic GSL content with a positive additive effect.

QTL associated with the aromatic GSL under control and salt stress conditions

In the case of the aromatic GSL NEO, two QTL were localized: NEO-1C on LG C3 and NEO-2C on C4. Both QTL have negative additive effects. These QTL account for 26% of the observed phenotypic variation. NEO-1C was mapped at *P* = 0.05. No QTL were mapped for indolic GSL under salt stress

QTL associated with the total GSL content under control and salt stress conditions

For total GSL content, three QTL, SUM-1C, SUM-2C and SUM-3C, were mapped on LGs: C7, C8 and C9, respectively. SUM-1C and SUM-2C show negative additive effects. SUM-2C has a positive additive effect. All of them together explain the percentage variation of 27%. Under salt stress, one QTL, SUM-1S, was localized on LG C7, which explains 10% of the phenotypic variation with a negative additive effect.

Table V-10: QTL detected at LOD > 2 under control treatment (C) for glucosinolate content µMol/gDM in *Brassica oleracea* mapping population Bo1TBDH. (QTL significant with *P* = 0.05 are marked bold).

Trait	Name of QTL	LG	Position (cM)	LOD	Interval	Flanking markers	Additive effect	Phenotypic variation explained (%)
IBE	**IBE-1C**	**C1**	**80**	**3.7**	**77 - 84**	**fito131 - pW220aH**	**-0.13**	**4.7**
IBE	IBE-2C	C5	79	5.2	72 -85	fito156a -pW164aE	-0.25	18.6
PRO	**PRO-1C**	**C3**	**11**	**4.3**	**10 - 13**	**pW212bE -fito272**	**0.44**	**15.6**
GNL	GNL-1C	C5	76	1.5	57 - 80	fito156a -pW164aE	-0.12	5.6
GNA	GNA-1C	C 3	7	1.8	6 - 15	pX141bH -pW212bE	-0.42	6.9
GNA	**GNA-2C**	**C 7**	**67**	**3.8**	**56 - 72**	**pW108aH -fito088b**	**-0.61**	**13.9**
GNA	**GNA-3C**	**C 9**	**68**	**10**	**58 - 78**	**fito016 -pW187bH**	**-0.95**	**32.9**
GBC	**GBC-1C**	**C 2**	**76**	**3.0**	**66 - 85**	**pW161aX -pW176aH**	**0.52**	**11.2**
GBC	GBC-2C	C 3	44	1.9	43 - 47	fito156c -pW125dE	0.48	7.4
GBC	**GBC-3C**	**C 9**	**21**	**2.4**	**14 - 25**	**fito163 -pX146cH**	**0.43**	**8.9**
NAS	**NAS-1C**	**C 4**	**24**	**2.0**	**21 - 37**	**pW205aH -pX105cE**	**-0.06**	**7.6**
NEO	**NEO-1C**	**C 3**	**96**	**4.0**	**91 - 101**	**BRMS017 -FC**	**-0.55**	**14.9**
NEO	NEO-2C	C 4	60	2.9	58 - 69	fito100c -pX130aD	-0.35	10.9
Aliphatic	**Aliphatic1C**	**C 7**	**56**	**4.9**	**50-65**	**BRMS042-pW108aH**	**-0.90**	**17.8**
Aliphatic	**Aliphatic2C**	**C 9**	**70**	**9.9**	**58-80**	**fito016 -pW187bH**	**-1.23**	**32.5**
Indolic	Indolic-1C	C 2	77	2.6	65-85	pW161aX - pW176aH	0.52	9.4
Indolic	**Indolic-2C**	**C 3**	**44**	**2.4**	**41 - 47**	**fito156c-pW125dE**	**0.59**	**8.8**
Indolic	Indolic-3C	C 9	20	2.1	14-25	pW256bH-fito163	0.43	7.7
SUM	**SUM-1C**	**C 7**	**66**	**1.95**	**53 - 72**	**pW108aH -fito088b**	**-0.79**	**7.4**
SUM	**SUM-2C**	**C 8**	**51**	**2.3**	**45 - 55**	**fito204a -pX130cD**	**0.81**	**8.6**
SUM	**SUM-3C**	**C 9**	**66**	**3.69**	**54 - 79**	**pW108gH - fito016**	**-1.0**	**13.5**

- Additive effect was calculated by subtracting TO1000DH3 alleles by Early Big alleles

Table V-11: QTL detected at LOD > 2 under salt treatment (S) for glucosinolate content µMol/ g DM in *Brassica oleracea* Bo1TBDH mapping population. (QTL significant with *P* = 0.05 are marked bold).

Trait	Name of QTL	LG	LOD	Position (cM)	Interval	Flanking markers	Additive effect	Phenotypic Variation explained (%)
IBE	IBE-1S	C2	1.2	90	85 -97	fito019 -fito375	0.06	4.9
PRO	**PRO-1S**	**C3**	**4.6**	**23**	**15 -31**	**fito272 -fito066**	**-0.26**	**15.8**
PRO	PRO-2S	C8	2.5	9	0 -18	pX103cD - fit040d	-0.17	9.8
RAA	**RAA-1S**	**C9**	**1.7**	**67**	**53 -84**	**fito016 - pW187bH**	**0.01**	**6.8**
GNA	**GNA-1S**	**C7**	**4.0**	**72**	**65 -78**	**fito088b - pX110aE**	**-0.52**	**15.3**
GBC	**GBC-1S**	**C9**	**4.6**	**21**	**15 -25**	**fito163 -pX146cH**	**0.31**	**17.4**
NAS	**NAS-1S**	**C4**	**1.5**	**68**	**63 -77**	**pX130aD - pW178bH**	**0.03**	**6.0**
4ME	**4ME-1S**	**C5**	**1.3**	**0**	**0 -10**	**fito389 - pW125aE**	**0.01**	**5.2**
Aliphatic	**Aliphatic-1C**	**C7**	**3.9**	**77**	**74-78**	**fito088b - pX110aE**	**0.60**	**14.5**
Indolic	**Indolic-1S**	**C9**	**4.6**	**20**	**15-25**	**pW256bH -fito163**	**0.32**	**16.8**
SUM	SUM-1S	C7	2.7	58	51 -72	BRMS042 - pW108aH	-0.61	10.4

- Additive effect was calculated by subtracting TO1000DH3 alleles by Early Big alleles.

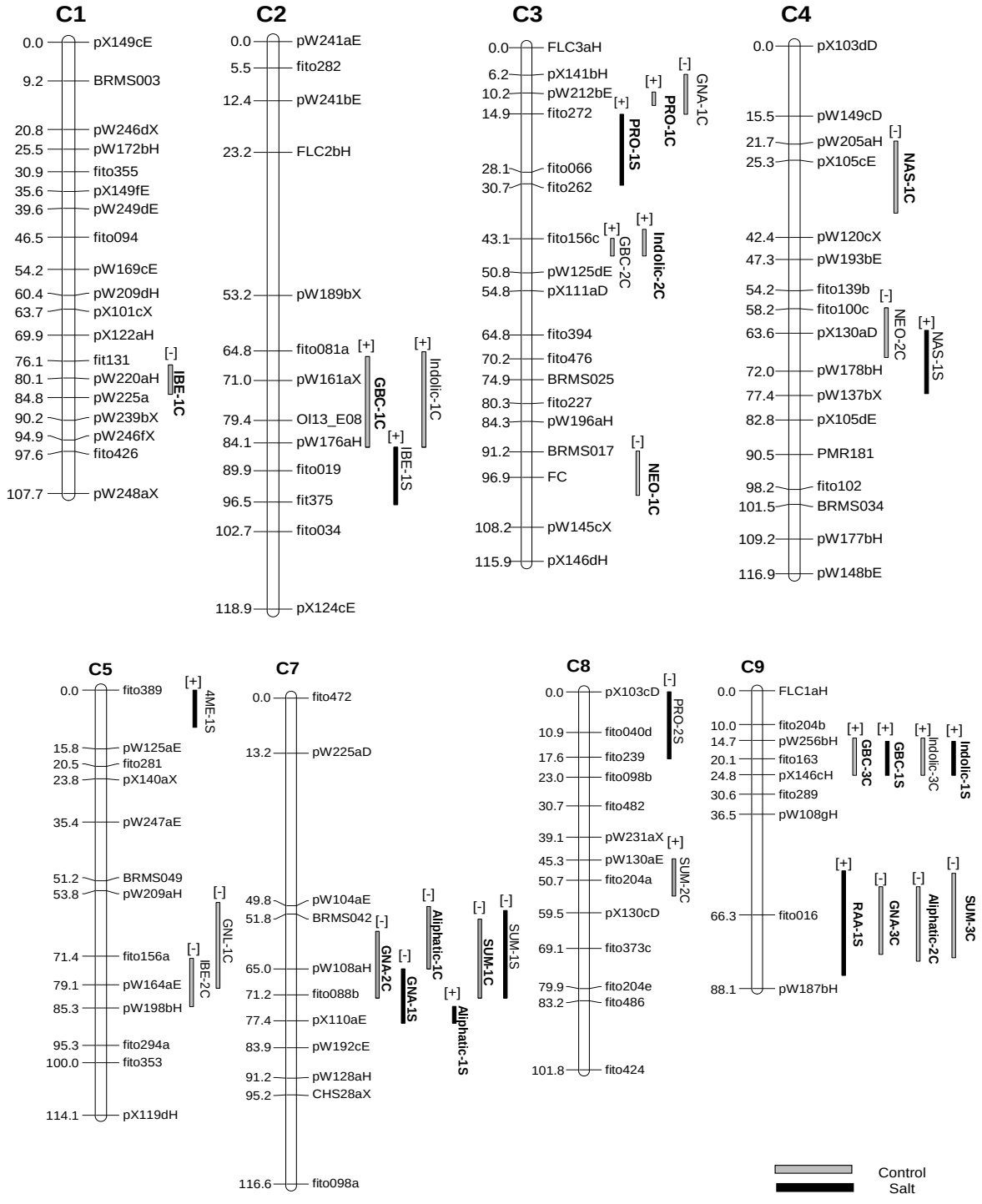


Figure V-11: Localization of QTL for glucosinolate in *Brassica oleracea* mapping population Bo1TBDH. (QTL significant with *P* = 0.05 are marked bold)

5.7 Discussion II

5.7.1 Variation of GSL under control and salt stress conditions

The parental lines exhibit a large contrast in GSL content. TO1000DH3 shows high GSL compared to Early Big under control and salt stress conditions. TO1000DH3 includes three aliphatic GSL components, IBE, PRO and GNA, as well as two indolic GSL constituents, GBC and NAS, and also one aromatic ingredient, NEO. Early Big includes one aliphatic component, GNL, two indolyl types, GBC and 4OH, and one aromatic one, NEO. These findings are consistent with those of Sotelo et al. (2014). In the same population they found only four types: RRA, GBC, NAS and NEO in the parent Early Big. A transgressive distribution was observed for the individual components and the total GSL content as well (Figure 10 and Appendix 13). This transgressive segregation states that the positive and negative alleles are dispersed in the two parents. In agreement with our findings, similar distribution patterns have been described in *B. juncea* (Ramchiary et al. 2007) and *B. oleracea* (Sotelo et al. 2014).

Under control conditions in the DH population, the indolic GSL dominated the other classes with 40% of the total GSL. This agrees with previous studies. Our results are also consistent with results reported in the same population (Sotelo et al. 2014), where indolic GSL were dominant in leaves. It should be noted that in our study, the leaves were harvested at 35 days after sowing. Similar results were found in kale leaves, where the concentration of indolic GSL was higher at the early vegetative growth stage 30 days after sowing (Velasco et al. 2007). Under salt stress, all GSL components and total GSL decreased. GBC represents the dominant component in all categories. These findings agree with those of Velasco et al. (2007), who found that GBC is the predominant constituent in *B. oleracea* leaves. Recently, a study by Issa et al. (2010) in another *B. oleracea* (AGDH), GBC was the prominent indolic GSL.

The correlations between the single components of the same category are mostly positive. The negative correlation between individual components belonging to different classes can be explained by the cross-talk between the biosynthesis routes of the different classes. This cross-talk between the different GSL classes was evidenced in different species of *Arabidopsis thaliana* (Gogishvili et al. 2007b, Berger et al. 2007) and *B. oleracea* (Issa et al. 2010).

RAA and 4ME data are lacking for both parents; however, they were detectable in the mapping population. In contrast to our results, Sotelo et al. (2014) found that ALY was undetectable in parents, but was measurable in the mapping population. This discrepancy might be due to the different growth conditions and the time of harvesting the plant material. This assumption was supported by the findings of Brown et al. (2003) in *Arabidopsis*, who found that the total GSL and composition in leaves varied with time.

Under salt stress, the aliphatic GSL are abundant relative to the other classes. This change can be attributed to the strong reduction in indolyl GSL and aromatic GSL relative to the weak reduction in aliphatic GSL. The cross-talk between the

biosynthesis of aliphatic and indolic GSL is evidenced in *Arabidopsis* (Gigolashvili et al. 2009), and in *Brassica rapa* ssp. *Pekinensis* (Kim et al. 2013). These authors reported that several transcription factors belonging to the cytochrome 450 family are involved in the biosynthesis of both the indolic and the aliphatic GSL. The reduction or blocking of the biosynthesis in the aliphatic GSL is accompanied by an increase in the indolic GSL and *vice versa* (Grubb and Abel 2006).

GBN, an aliphatic GSL, was detected only under salt stress, which might be due to the hydroxylation of GBN to GNL being suppressed by salinity, resulting in GBN accumulation. This speculation is advocated by the findings of (Yan and Chen, 2007), who found that under optimal growth conditions the hydroxylation step is accelerated due to the availability of nitrogen. Thus, this decline in the aliphatic hydroxylated forms may be ascribed to nitrogen-deficiency as a consequence of salt stress. The most well-known antioxidant RAA slightly decreased under salt stress. Conversely, Guo et al. (2013) observed a 2.1-fold increase in sulforaphane, one of the products of RAA hydrolysis under stress of 100 mM NaCl in *Brassica oleracea* var. italica cv. Youxiu. Similarly, we found a significant increase in RAA in a mapping population of *B. napus* Mansholts and Samourai under stress of 200 mM NaCl.

The common diminution pattern of GSL individuals under salt stress suggests co-ordinate biosynthesis regulation. In *Arabidopsis*, Hirai et al. (2005) found that the genes that underlie the biosynthesis of GSL are co-regulated. This author suggests that one master mechanism controls the expression of these genes. This significant reduction in concentration of most GSL components and total GSL might be due to leakage of GSL from vacuoles into cytoplasm, where they are hydrolyzed by myrosinase (Pang et al. 2012). Another explanation is that the hydrolysis of GSL releases the inorganic elements, such as Sulfur and Nitrogen that are included in the GSL structure. The released elements are harnessed at earlier stages in the metabolic process, such as protein biosynthesis (Falk et al. 2007).

5.7.2 QTL analysis and localization

A total of 32 QTL were mapped, 21 under control conditions and 11 under salt stress. Based on the genetic comparative studies conducted to identify the homeologous regions between *B. oleracea*, *B. rapa* and *Arabidopsis* (Lukens et al. 2003, Iñiguez Luy et al. 2009), our aim has been to identify the genes that might be included in the synthesis of GSL.

Mapping QTL, a number of hotspots were identified, two on LG C9 and one on LG C7. At the top of LG C9, four QTL show co-localization: two QTL for GBC and two QTL for the sum of indolic GSL. In the case of GBC, one QTL is control-specific and the other salt-specific, as is the sum of total GSL. Furthermore, the additive effects of these QTL are positive, indicating that the alleles were transmitted from Early Big, the parent with high indolic content. These alleles are in a couple phase, as expected, as they are positively correlated, particularly under salt stress. This positive correlation is foreseeable because GBC is the major component of the indolic GSL class. At the lower part of LG C9, another cluster comprising four QTL was mapped. All of the QTL

are aliphatic-GSL-specific. Three of them are control-specific and one is salt-specific. The three control-specific QTL show negative additive effects, while the salt-specific one exhibits a positive additive effect. The antithetical additive effects indicate that the alleles that control the biosynthesis routes are in a repulsion phase. The co-localization of these four QTL is plausible because all of them are associated with methionine-derived GSL. Moreover, RAA is the precursor of GNA. These results point to the presence of a BoGSL-ALK gene modulating the biosynthesis of GNA from RAA through desaturation and the loss of methylsulphinyl core (Li and Quiros 2003). Supporting this notion, this region revealed homology with At4, where the GSL-ALK was mapped in *Arabidopsis* (Mithen et al. 1995). The identification of these genes, especially GS-ALK, is of great importance. The deactivation of GS-ALK might be helpful in producing *Brassica* cultivars with high RAA content (Li and Quiros 2003, Wentzell et al. 2007).

On LG C7, six QTL related only to aliphatic GSL were detected. The QTL GNA-1S and Aliphatic-1S revealed tight overlapping. This is expected because GNA represents the major component of the aliphatic GSL class. Based on the synteny data proposed by (Iñiguez-Luy et al. 2009), this genomic region shows collinearity with the *B. rapa* LG A7, where the QTL for GNA was mapped (Lou et al. 2007) and with the *B. oleracea* mapping population (AGDH) where one QTL for GNA was mapped on LG C7 (Issa et al. 2010). The total GSL content of QTL SUM-1C and SUM-1S revealed a complete overlap, meaning that one master mechanism is controlling the GSL biosynthesis under control and salt stress.

Two QTL were mapped at the top of LG C3, one for GNA and one for PRO. Our results are in agreement with those of Sotelo et al. (2014) for the same population, where these QTL were mapped at the same position. Consistent with our findings, in another *B. oleracea* mapping population (AGDH) on C3, one QTL for GNA and one for PRO were also mapped (Issa et al. 2010). This region shows homology with the top of *Arabidopsis* chromosome 5 (At5) and bottom of chromosome 2 (At2). The genes MAM 1 and MAM2 were identified at the top of At5 and the GSL-OH gene was mapped at the bottom of At2 (Kliebenstein 2009). Moreover, an orthologous to MAM 1 was identified in *B. oleracea* (Bol-ELONG). This gene also accounts for the formation of 4-carbon GSL in *Arabidopsis* and *B. oleracea* (Li and Quiros 2003). All genes in this family have the same structure, direction and function as those in *Arabidopsis* and *B. oleracea* (Gao et al. 2006). Based on these results, we can conclude that this region harbors MAM 1 and MAM2, which are involved in the biosynthesis of GNA. GNA undergoes further modification with GSL-OH to produce the hydroxylated alkenyl PRO. In another study, GSL-OH was mapped on LG C9 close to the GSL-ALK gene (Gao et al. 2007). Most likely, there are many homologous genes scattered on different LGs. It is well known that triplicates from each *Arabidopsis* gene are expected to occur in the *B. oleracea* genome.

On LG C2, two QTL for GBC and total indolic GSL content were mapped. The QTL for GBC content in seeds was identified at the same position (Sotelo et al. 2014). By an *in silico* analysis of *Arabidopsis,* Sotelo et al. found that the gene CYP79B2 was in the

interval of the QTL specific for total indolic GSL in seeds. These findings indicate that these genes are not tissue-specific. Similarly, in our study on *B. napus* mapping population of Mansholts and Samourai, QTL for leaf GSL co-localized with the QTL for seed, which had been mapped earlier in other studies. This is indicative for a master mechanism of GSL biosynthesis; GSL might be synthesized in vegetative organs and translocated later into seeds.

Conclusion

In may be concluded that salt stress decreases the GSL content and profile in parental lines and in the DH mapping population as well. Based on synteny studies, we propose the presence of a number of causal genes that were identified earlier as being involved in the different types of GSL biosynthesis.

Several reasons are thought to lie behind the reduction in total GSL under salt stress. Under salt stress, plants tend to reduce the biosynthesis of some metabolites, among them GSL (Pang et al. 2012, López-Berenguer et al. 2009). This supposition is supported by the findings of Steinbrenner et al. (2012) in *B. rapa,* who found that temporal stresses like salinity, drought and nutrient deficiency delayed the accumulation of some metabolites like GSL. The reduction in GSL under salt stress might result from leakage of GSL from the vacuole to the cytosol, where they are hydrolyzed by myrosinase (Pang et al. 2012). Additionally, it has been reported that GSL are reservoirs for nutrients, like Nitrogen and Sulfur. One of the effects of salinity is nutrient deficiency; therefore, plants hydrolyze GSL and benefit from the released elements as an alternative, so as to ensure the primary stages of metabolism such as protein biosynthesis (reviewed by Martinez-Ballesta et al. 2013).

More work would be helpful to reach a broader view and clearer insights into the effect of salinity on leaf GSL composition and content of *B. oleracea*. The availability of the complete genome sequence of *B. oleracea* will pave the way for the identification of the causal genes that control GSL variation. Importantly, the parental line, TO1000DH3 is the reference for the full genome sequence project, and the second parent, Early Big has been included in several studies to identify the causal genes involved in the biosynthesis of GSL.

Chapter VI

General discussion

The present study investigates the effect of salinity on seed germination and on the young plant stage in doubled-haploid (DH) mapping populations of *B. napus* and *B. oleracea*. Furthermore, the effect of salinity on glucosinolate (GSL) in these populations was addressed.

6.1 Effect of salinity on seed germination

The effect of salinity on seed germination is investigated in two DH populations of *B. napus,* Alesi × H30 and Mansholts × Samourai, and in one DH population of *B. oleracea,* Bo1TBDH. The saline conditions involved treatments with 200 mM NaCl in *B. napus* and 100 mM NaCl in *B. oleracea*. The germination parameters, germination percentage (G%), germination pace (GP) and salt tolerance index (STI) were analyzed. A wide variation was observed in all populations, especially under salt stress. Salt stress reduced seed germination significantly, and slowed down the germination rate in all populations. Mostly, the distribution of traits was normal, with positive and negative transgressive segregation, but several DH lines showed performances better than the parent with high traits values and other DH lines revealed trait values lower than the low parent. The quantitative trait loci (QTL) analysis resulted in the identification of several QTL for all studied traits in all populations. A number of these QTL were adaptive, and were mapped under either stress or control conditions. Additionally, constitutive QTL were detected that control variations in the respective traits under both growth conditions. The constitutive QTL indicate that some genomic regions harbor genes that control seed germination under both control conditions and salt stress. The adaptive QTL show that some genomic regions hold genes that underlie the variation of traits under either salt or control conditions. Both QTL groups are important for the selection of salt-tolerant DH lines in a marker-assisted selection. The significant reduction in G%, GP and STI is induced by osmotic stress and ion toxicity due to excess ions in the germination medium surrounding the seeds or to the accumulation of ions inside the seed tissues.

6.2 Effect of salinity on plant growth

The genotypic variation under salt stress of plant growth at the young plant stage was measured in the *B. napus* DH population of Mansholts and Samourai and the *B. oleracea* Bo1TBDH population. Salt treatments comprised 200 mM NaCl for the *B. napus* population and 100 mM NaCl for the *B. oleracea* population.

A significant variation was observed in all traits in both populations. In both populations, salinity reduced plant growth. The reduction in FW and DW was lose, while RWC showed a smaller reduction. This reduction resulted from the negative effect of osmotic stress induced by the high concentration of Na^+ and Cl^- ions surrounding the plant root, which impairs water uptake. Another contributing factor may be the ion toxicity from accumulation of ions in the plant tissues. The imbalance between Na^+ and Cl^- ions on the one hand and other ions causes nutritional deficiency

by hindering the uptake of essential nutrients such as Ca^{2+}, Mg^{2+} and K^+. This notion is supported by the negative and significant correlation between Na^+, FW and DW. In both populations, the remaining parameters exhibit an increase under salt stress; the exception is K^+ in the *B. olercea* population. SPAD values show an increase under salt stress; this increase in SPAD values might be ascribed to the reduction in leaf area, which increases the number of chloroplasts per square centimeter. A dramatic increase was observed in the sodium-related traits, Na^+ mg/ g DM and Na^+/ K^+. Unexpectedly, K^+ increased in the *B. napus* DH population, which might be due to the ability of plants to maintain high K^+ content so as to employ it as osmoticum or a coenzyme. Conversely, K^+ content showed a reduction in *B. oleracea* population, though the NaCl concentration was lower than that applied for *B. napus*. This is expected under salt stress where Na^+ competes with K^+ to enter the cell through K^+-transporting channels. The maintenance of high K^+ concentration under salt stress is one of the salt tolerance parameters; therefore, with the findings of other groups these findings confirm the superiority of the amphidiploids species over their diploid ancestors.

In both populations, several QTL were mapped under control versus salt stress conditions. In the case of the *B. napus* DH population, groups of QTL hotspots were mapped to different linkage groups (LGs). The largest hotspot was localized on LG C3, where many traits clustered together. In other words, one genomic region harbors the gene(s) that regulate(s) the variation of more than one trait under both control and salt stress conditions. The variation of in these traits might be controlled by the pleiotropic effect of one gene, or by different, closely linked genes controlling variations independently. On LG A1, two QTL, i.e. SPAD-1S and SPAD-2S, were mapped under salt stress and found clustered together. These are adaptive QTL, since they were mapped only under salt stress. The QTL that were identified under both growth conditions, like SPAD-2C and SPAD-3S, are constitutive QTL. Likewise, in the case of the *B. oleracea* population, QTL hotspots were observed on LG C3, where there is an overlap between the intervals of QTL FW-3C, FW-2S, DW-1C, K-2C and K-1S. This overlapping between the QTL suggests that a common mechanism controls the variation in these traits under different growth conditions. These QTL hotspots add weight to these genomic regions, because more than one desirable trait can be improved jointly. Interestingly, QTL for Na^+/K^+ were mapped in both populations on LG C9. This indicates the presence of gene(s) that control(s) Na^+/K^+ uptake.

Conclusion

We can conclude that salt stress causes a reduction in plant growth either at the seed germination or at the young plant stage. In both populations, a reduction in the FW, DW and RWC was observed. Reduction in these parameters is associated with an increase in other constituents, like Na^+ content and SPAD values. The most plausible explanation for this decline in FW, DW and RWC is the osmotic stress imposed by the NaCl solutions or by the ion toxicity due to the build-up of Na^+ and Cl^- ions. In both populations, two types of QTL constitutive and adaptive QTL were mapped. Constitutive QTL are not environment-specific; they govern the trait(s) variation(s) under both control and salt conditions, while the adaptive QTL govern the variation of

traits under one environment, either control or salt conditions. Interestingly, QTL for Na^+/K^+ were mapped in both populations on LG C9. This indicates the presence of gene(s) that control(s) Na^+/K^+ uptake. Identification of the QTL hotspots adds significance to the genomic regions where they were mapped because more than one desirable trait can be improved jointly.

6.3 Glucosinolates' variation under control and salt stress conditions

Variation in leaf GSL was studied under the same growth conditions in both the *B. napus* and *B. oleracea* DH populations. In the case of the *B. napus* DH population of Mansholts and Samourai, Mansholts exhibits high GSL content under control conditions and salt stress compared with Samourai. Under salt stress, the two parents perform differently. Mansholts reveal an increase, while Samourai exhibits a drop in total GSL content. The DH population exhibits a reduction in total GSL content. This reduction is driven by a reduction in the aliphatic GSL. In the DH population and the parental lines, the aliphatic GSL type was predominant compared with the indolic and aromatic GSL types under control or salt stress. Under salt treatment in the DH population, there was an increase in the indolic and aromatic GSL and a fall in the aliphatic GSL, which may be attributed to cross-talk between the different biosynthesis pathways of the groups. All single components reveal a decline under salt stress, except RAA and GBC. Because of their antioxidant properties the increase in both RAA and GBC components might occur in order to detoxify the reactive oxygen species (ROS) induced by salt stress. Mostly, the GSL components belonging to the same class correlate positively and significantly under control and salt stress.

In the context of QTL mapping, several QTL were mapped under control and salt stress conditions in the *B. napus* population. Noteworthy is that a QTL hotspot was localized where QTL for seed GSL had been mapped earlier. This was clear, especially on LG A9, where there are five QTL for the aliphatic types PRO, RAA and GNA, in addition to one QTL for total aliphatic GSL and one for total GSL. This finding suggests that in this genomic region many genes are included in the biosynthesis of aliphatic GSL in seeds and leaves. Another large hotspot was detected on LG C2, where the second major QTL for seed GSL was identified. This hotspot includes QTL for aliphatic, indolic and aromatic GSL. There are probably genomic regions that harbor genes to control variations in the three major classes. Most likely, these genes are involved in the core structure of the biosynthesis stage. There are QTL that control GSL variation under either control or salt stress conditions, while other QTL control variation under both control and salt conditions. The correlations between the aliphatic GSL components are positive and significant, as also between the indolic GSL individuals. This is fully expected, because the components of each class have a common precursor.

In the case of the *B. oleracea* Bo1TBDH population, the two parents show a large variation in GSL components and concentrations. The parent TO shows a high GSL content relative to the parent EB. All of the single components reveal a reduction under salt stress, except GBC. Furthermore, GBN was detected under salt stress only. Under control and salt stress conditions in TO and in the DH population, PRO and

GBC represented the predominant aliphatic and indolic GSL, respectively. Unfortunately, due to the poor germination of EB we could not analyze its GSL under salt stress. Three QTL hotspots were identified; two on LG C9 and one on LG C7. At the top of C9, four QTL show co-localization: two QTL for GBC and two QTL for the sum of indolic GSL. The clustering of these QTL is expected, because GBC is the major component of the indolic class. Similarly, four QTL were mapped on the lower part. All of them were specific for aliphatic GSL components.

6.4 QTL for seed germination versus QTL for plant growth at the young stage

No coincidence was found between the positions of the QTL for seed germination and those QTL for plant growth. This result indicates that different genomic regions control salt tolerance at different growth stages. These findings are in accord with results reported earlier in other crops, like tomato (Foolad and Chen 1999) and barley (Mano and Takeda 1997). In the case of the *B. napus* DH population, all QTL for germination were localized on separate LGs where no QTL for growth stage was mapped. The only exception was one QTL for growth was mapped with QTL for seed germination on LG A9. Nevertheless, there was no overlap between the growth-related QTL with the germination-related QTL. As for the *B. oleracea* DH population Bo1TBDH, almost no overlap was found, though germination-related QTL shared the same LGs with QTL for growth traits (Figures 1, and 2).

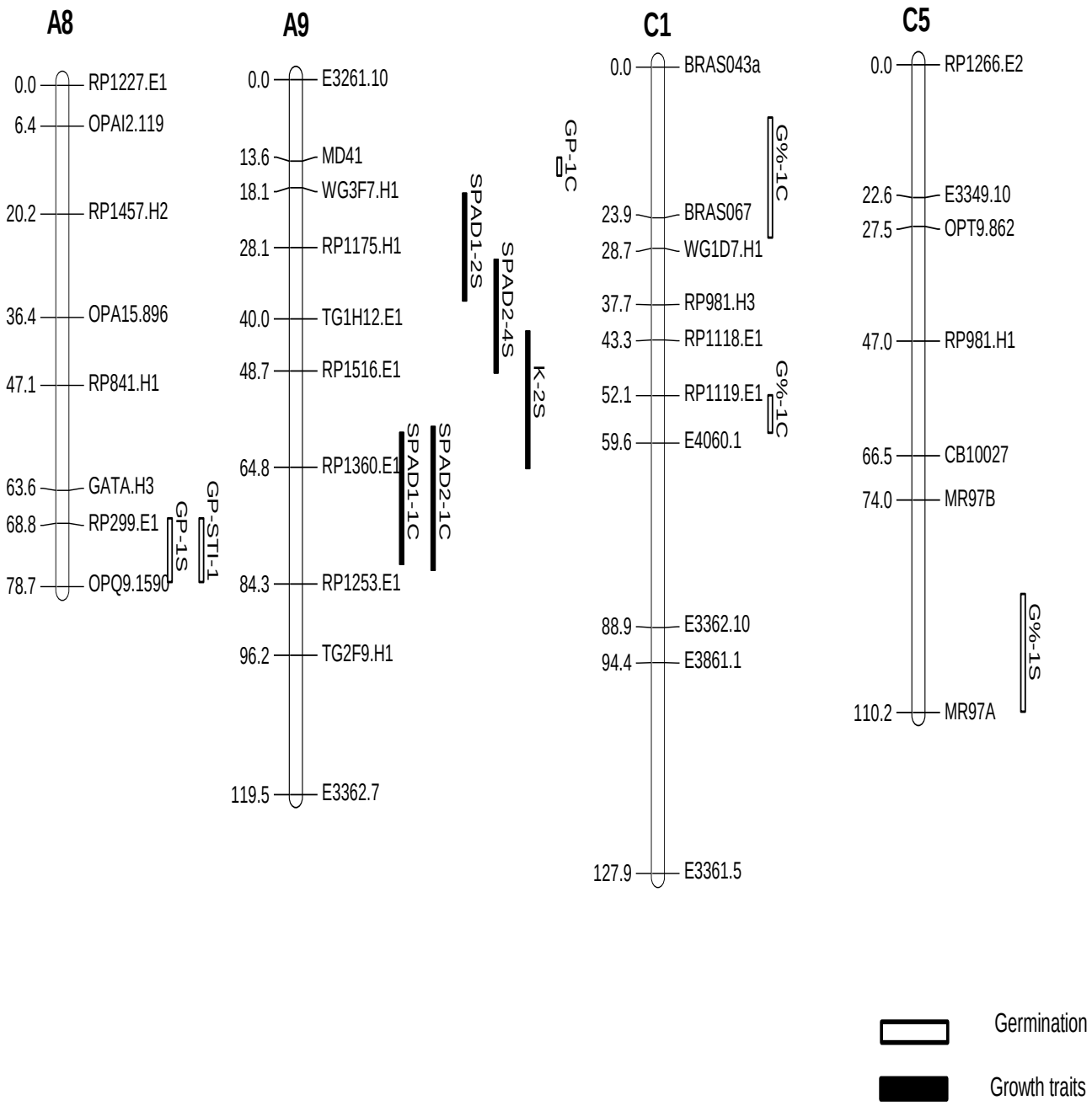


Figure 1: The relation between QTL for germination and QTL for growth traits in *B. napus* DH population Mansholts × Samourai.

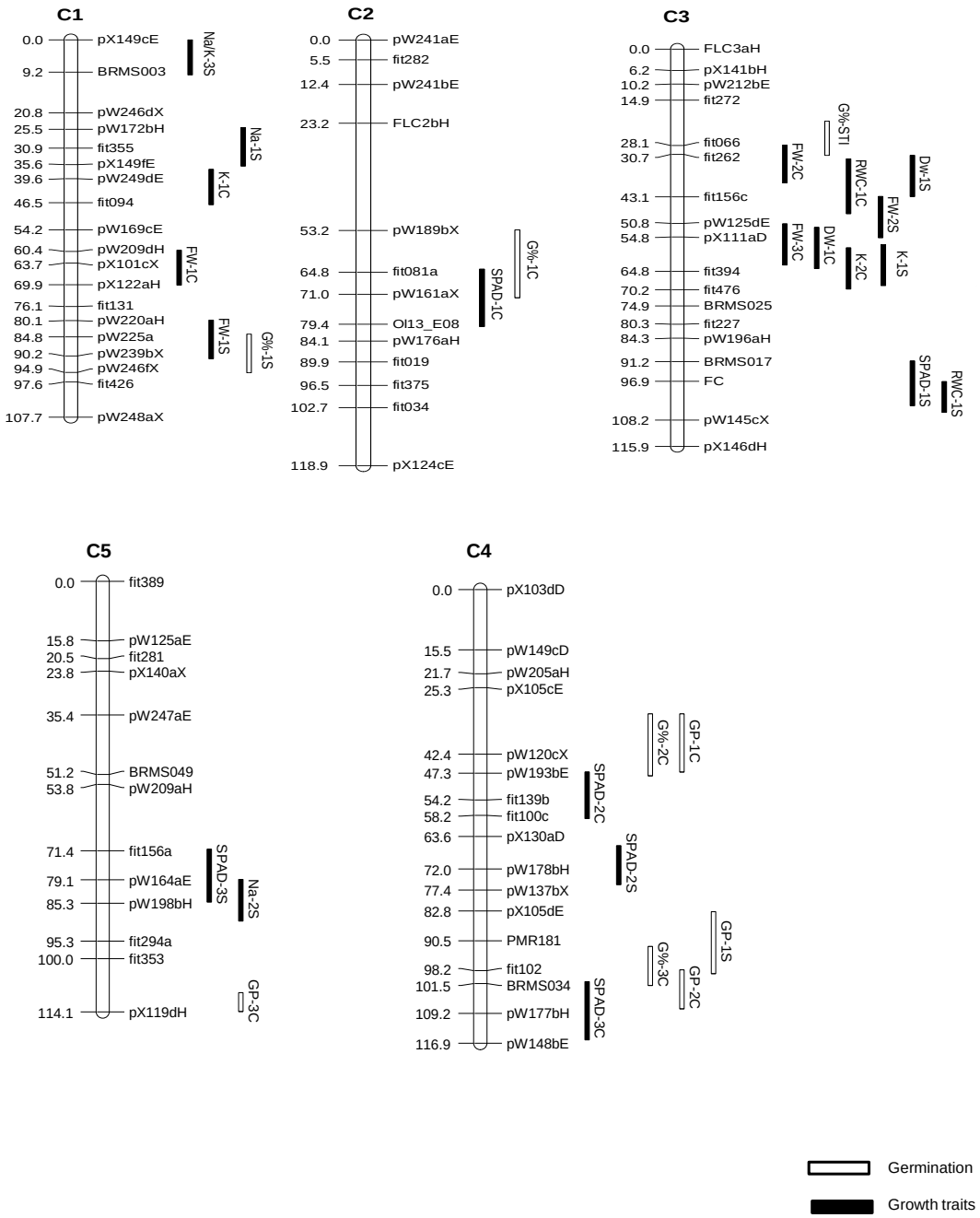


Figure VI-2: The relation between QTL for germination and QTL for growth traits in the *Brassica oleracea* DH population Bo1TBDH

Summary

Brassica species are of great importance for human food and animal feed supply. *Brassica napus* occupies the second position among the oilseed crops behind soybean. *Brassica oleracea* includes numerous species of vegetables. Salinity is one of the abiotic stresses that adversely affect the productivity of these crops globally. Salinity tolerance varies along the plant ontogeny, meaning that it is stage-related, species-specific and organ-specific. In comparison to our knowledge about the genetic properties of seed glucosinolates (GSL), our knowledge about the genetic control of leaf GSL is rather limited.

The objectives of this project are: (1) to study the effect of salinity on two stages of plant growth, namely seed germination and the young plant stage, and to map QTL (Quantitative Trait Loci) that control salt tolerance in both growth stages in doubled-haploid (DH) mapping populations of *B. napus* and *B. oleracea*, (2) to examine the variation in leaf GSL content and the impact of salinity on GSL, and to map the QTL that control GSL variation under control and salt stress.

The effect of salinity on seed germination was investigated in three double haploid (DH) populations, two *B. napus* populations and one *B. oleracea* population. The first two were derived from Alesi × H30 and from Mansholts × Samourai, respectively. The *B. oleracea* population was derived by crossing a DH rapid cycling TO1000DH3 (TO) line and a DH broccoli line Early Big (EB). 138 DH lines for each population were tested either for germination experiments or for the greenhouse experiments. The frame work maps that were used for QTL mapping compromised 188, 208 and 128 markers Alesi × H30, Mansholts × Samourai and TO × EB, respectively.

The salt treatments were 200 mM NaCl for the *B. napus* populations and 100 mM NaCl for the *B. oleracea* population. Ten seeds from each DH line were sown in nine cm Petri dishes on filter paper moistened with five ml tap water for control and five ml solution of the corresponding salt concentrations. The Petri dishes were incubated at 20°C in the dark. The number of germinated seeds was counted daily. The results revealed that salt stress significantly reduced seed germination and slowed down the germination rate. Nevertheless, several DH lines showed a better performance than the parent, with a high germination percentage and high germination pace (germination rate) in all populations. Several QTL were mapped for all the studied traits in all populations. A number of these QTL control the variation in these traits under both control and salt stress conditions, while others control the trait variations under either the control or salt stress.

The influence of salinity on plant growth at the young plant stage was evaluated in the *B. napus* DH population Mansholts × Samourai and the *B. oleracea* population TO × EB. The DH lines of each population and their parents were tested in pot experiments in the greenhouse under semi-controlled conditions in two replicates. The salt treatments were 200 mM NaCl for the *B. napus* populations and 100 mM NaCl for the *B. oleracea* population. A number of traits, i.e. fresh weight (FW), dry weight (DW), chlorophyll content (SAPD), relative water content (RWC), sodium content (Na^+ mg/ g

DM), potassium content (K^+ mg/ g DM), and sodium potassium ratio (Na^+/ K^+), were scored. The salt stress started on 21 days after sowing (das) for two weeks, the experiments were terminated on 35 das.

A significant variation was observed in all traits in both populations. In both populations salinity reduced plant growth, where a reduction in FW and DW was very large, while RWC showed a weak reduction. In both populations, the remaining parameters exhibited an increase under salt stress; the exception was K^+ in the *B. oleracea* population. SPAD values showed an increase. Similarly, Na^+ mg/ g DM and Na^+/ K^+ exhibited a very large increase. Unexpectedly, K^+ increased in the *B. napus* DH population. Oppositely, the K^+ content decreased in *B. oleracea* population. Maintenance of high K^+ concentration under salt stress is one of the salt tolerance characteristics. These findings supporting the preceding results that revealed *B. napus* was more salt tolerant than *B. oleracea*.

In both populations, several QTL were mapped under control and salt stress. In the *B. napus* DH population, a number of QTL hotspots were mapped on different linkage groups (LGs). The largest hotspot was localized on LG C3. Likewise, in the *B. oleracea* population, QTL hotspots were detected on LG C3, where many traits cluster together. In other words, one genomic region harbors gene(s), which regulate(s) the variation of more than one trait under both control and salt stress conditions. The variation in these traits might be controlled by the pleiotropic effect of one gene or by different genes controlling their variation independently. The genomic regions, where QTL for more than one trait were mapped, are of great importance because more than one trait can be improved jointly.

Leaf GSL variation was investigated under the same growth conditions in both *B. napus* and *B. oleracea* DH populations. In both populations, the parental lines varied largely in their GSL profiles and contents. In the *B. napus* population, Mansholts exhibited high GSL content under control and salt stress conditions compared with Samourai. The two parents performed differently under salt stress, Mansholts revealed an increase, while Samourai exhibited a reduction in the total GSL content. In the *B. oleracea* population, the parental line TO showed higher GSL content than the parent EB. In both DH populations, all components revealed a decline under salt stress, except RAA and GBC in the *B. napus* population and GBC in the *B. oleracea* population. Because of their antioxidant properties, the increase in RAA and GBC might serve to detoxify the effects of ROS, which are produced as a consequence of salt stress. Several QTL were mapped under control and salt stress. Noteworthy, in the *B. napus* mapping population, QTL hotspots were mapped where QTL had been mapped earlier for seed GSL. This was clear, especially on LG A9 and LG C2. In the *B. oleracea* mapping population, QTL hotspots were localized on LG C9 and LG C7. The clustering of these QTL in both populations was logical because occasionally they were QTL for GSL components of the same class.

No consistency was found between the QTL controlling seed germination under salt stress and the QTL that control the trait variations at the young plant stage. These

results suggest that different mechanisms control salt tolerance throughout the plant life cycle. By combining QTL for salt tolerance of different developmental stages a good adaptation to salt stress can be achieved.

Zusammenfassung

Brassica-Arten sind von großer Bedeutung für die menschliche Ernährung und für die Tierernährung. *Brassica napus* ist hinter der Sojabohne die zweit wichtigste Ölsaat. *Brassica oleracea* umfasst mehrere wichtige Gemüseformen. Die Produktivität dieser Arten wird weltweit durch Versalzung als ein biotischer Stressfaktor beeinträchtigt. Die Salztoleranz ist abhängig von der Pflanzenentwicklung, also stadienspezifisch, artspezifisch und organspezifisch. Im Vergleich zu unseren Kenntnissen über Samenglucosinolate ist wenig über die genetische Kontrolle von Blattglucosinolaten bekannt.

Die Arbeit hat folgende Zielsetzungen: (1) den Einfluss von Salzstress auf zwei Entwicklungsstadien zu untersuchen, und zwar auf die Keimung und die Jungpflanzenentwicklung, und in doppel-haploiden (DH) Populationen von *B. napus* und *B. oleracea* QTL (Quantitative Trait Loci) für Salztoleranz in beiden Entwicklungsstadien zu kartieren, und (2) die Variation im Blatt-GSL Gehalt zu untersuchen und QTL zu kartieren für den GSL Gehalt in einer Kontrolle und unter Salzstress.

Der Einfluss von Salzstress auf die Keimfähigkeit wurde an drei DH Populationen untersucht, zwei *B. napus* und eine *B. oleracea* Population. Die beiden erstgenannten Populationen wurde aus Alesi × H30 bzw. Mansholts × Samourai entwickelt. Die *B. oleracea* Population entstand aus der Kreuzung zwischen der „rapid cycling“ Linie TO1000DH3 (TO) und einer DH Linie aus dem Broccoli Early Big (EB). Die Anzahl DH Linien 138 Für die QTL Analysen wurde eine framework map verwendet mit 188, 208 bzw. 128 Markern für die Populationen Alesi × H30, Mansholts × Samourai bzw. TO × EB.

Die Versuche wurden mit den Salzkonzentrationen 200 mM NaCl für *B. napus* und 100 mM NaCl für *B. oleracea* durchgeführt. Von jeder DH Linie wurden 10 Samen in 9 cm Petrischalen auf Filterpapier ausgelegt, das mit 5 ml Leitungswasser als Kontrolle bzw. 5 ml Salzlösung befeuchtet war. Die Petrischalen wurden bei 20 °C im Dunkeln inkubiert. Die Anzahl gekeimter Samen wurde täglich gezählt. Unter Salzstress war die Keimfähigkeit signifikant reduziert und die Keimgeschwindigkeit verlangsamt. Einige DH Linien übertrafen dabei ihre Eltern mit einer höheren Keimfähigkeit und Keimgeschwindigkeit. Es konnten für alle untersuchten Merkmale mehrere QTL identifiziert werden. Einige dieser QTL beeinflussen die Merkmalsausprägung sowohl in der Kontrolle als auch unter Salzstress, während andere QTL nur entweder in der Kontrolle oder in der Stressvariante auftreten.

Der Einfluss von Salz auf die Jungpflanzenentwicklung wurde in der *B. napus* DH Population Mansholts × Samourai und in der *B. oleracea* Population TO × EB untersucht. Von jeder Population wurden die DH Linien und die Eltern in Topfversuchen im Gewächshaus bei halbkotrollierten Bedingungen angezogen. Die Salzkonzentrationen waren 200 mM NaCl für *B. napus* und 100 mM NaCl für *B. oleracea.* Die erfassten Merkmale waren Frischgewicht (FW), Trockengewicht (DW), Chlorophyllgehalt (SPAD), relativer Wassergehalt (RWC), Natriumgehalt (Na^+ mg/g DM), Kaliumgehalt (K^+ mg/g DM) sowie das Natrium/Kalium-Verhältns (Na^+/K^+). Der

Salzstress begann 21 Tage nach Aussaat und der Versuch wurde 35 Tage nach Aussaat beendet.

In beiden Populationen trat für alle Merkmale eine signifikante Variation auf. In beiden Populationen war das Wachstum unter Salzstress gehemmt, wobei FW und DW sehr stark reduziert waren während der RWC nur eine leichte Reduktion zeigte. Die anderen Merkmale zeigten unter Salzstress einen Anstieg mit Ausnahme von K^+ in der *B. oleracea* Population. Die SPAD Werte zeigten einen Anstieg. Auch Na^+ mg/g DM und Na^+/K^+ zeigten einen starken Anstieg. Der K^+ Gehalt stieg in der *B. napus* Population unerwartet an, während er in der *B. oleracea* Population abnahm. Das Aufrechterhalten von hohen K^+ Konzentrationen unter Salzstress ist ein Merkmal für Salztoleranz. Diese Ergebnisse unterstützen frühere Ergebnisse dass *B. napus* eine höhere Salztoleranz hat als *B. oleracea*.

In beiden Populationen wurde sowohl in der Kontrolle als auch unter Salzstress eine Reihe von QTL identifiziert. In der *B. napus* Population wurden auf verschiedenen Kopplungsgruppen (LG) QTL „hotspots“ entdeckt. Der größte „hotspot“ lag auf LG C3. Auch in der *B. oleracea* Population wurde auf LG C3 ein „hotspot“ entdeckt mit QTL für mehrere Merkmale. Hier liegen also in derselben Region des Genoms Gene für mehr als nur ein Merkmal sowohl in der Kontrolle als auch unter Salzstress. Die Variation dieser Merkmale wird entweder von einem Gen mit pleiotropem Effekt gesteuert oder von mehreren unabhängigen Genen. Regionen mit QTL für mehrere Merkmale sind von großem Interesse weil dadurch mehr als nur ein Merkmal gleichzeitig verbessert werden kann.

Unter den gleichen Bedingungen wurde in den beiden *B. napus* bzw. *B. oleracea* Populationen auch die Variation in den Blatt-GSL untersucht. Die Elternlinien beider Populationen unterschieden sich stark in GLS Gehalt und Zusammensetzung. In der *B. napus* Population hatte Mansholts einen im Vergleich zu Samourai hohen GSL Gehalt sowohl in der Kontrolle als auch unter Salzstress. Unter Salzstress verhielten sich die beiden Eltern unterschiedlich, Mansholts zeigte einen Anstieg und Samourai eine Abnahme des GSL Gesamtgehalts. In der *B. oleracea* Population zeigte die Elternlinie TO eine höheren GSL-Gehalt als der Elter EB. Die einzelnen GSL Komponenten zeigten in beiden DH Populationen eine Abnahme bei Salzstress mit Ausnahme von RAA und GBC bei *B. napus* und GBC bei *B. oleracea*. Aufgrund ihrer antioxidativen Eigenschaften könnte der Anstieg von RAA und GBC dazu dienen die ROS zu detoxifizieren, die als Reaktion auf Salzstress produziert wurden. Mehrere QTL wurden sowohl in der Kontrolle als auch unter Salzstress kartiert. In der *B. napus* Population wurden QTL „hotspots“ in Regionen identifiziert in denen bereits früher QTL für Samen-GSL lokalisiert wurden. Dies war vor allem auf LG A9 und LG C2 der Fall. In der *B. oleracea* Population wurden QTL „hotspots“ auf LG C9 und LG C7 lokalisiert. Das gleichzeitige Auftreten von QTL an derselben Position lässt sich teilweise dadurch erklären dass es sich um Komponenten derselben GSL Gruppen handelt.

Es gab keine Übereinstimmung zwischen den QTL für die Keimfähigkeit unter Salzstress und den QTL für Variation bei Jungpflanzen. Dies weist darauf hin dass für die Salztoleranz während der Pflanzenentwicklung unterschiedliche Mechanismen verantwortlich sind. Durch die Kombination von QTL für Salztoleranz in den unterschiedlichen Entwicklungsstadien lässt sich eine verbesserte Anpassung an Salzstress erreichen.

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Appendices

Appendix 1

Mean, minimum, and maximum of germination percentage, germination pace, and respective salt tolerance indices determined in DH populations of a) *B. napus* Alesi × H30, b) *B. napus* Mansholts × Samourai and c) *B. oleracea* Bo1TBDH populations under control and salt treatment.

Population	Ranges	Germination %		Germination pace		G% STI	GP STI
		Control	Salt	Control	Salt		
a) Alesi × H30							
	Minimum	90.0	10.0	0.2	0.1	7.1	34.4
	Maximum	100.0	100.0	0.6	0.3	100.0	81.0
	Mean	99.6	74.9	0.3	0.1	74.5	56.2
b) Mansholts × Samourai							
	Minimum	90.0	0.0	0.2	0.0	0.0	0.0
	Maximum	100.0	100.0	0.6	0.3	100.0	100.0
	Mean	89.03	74.8	0.4	0.2	75.2	47.8
C) Bo1TBDH							
	Minimum	60.0	0.0	0.1	0.0	0.0	0.0
	Maximum	100.0	100.0	0.4	0.3	100.0	100.0
	Mean	98.7	78.4	0.2	0.2	76.5	72.1

Appendix 2

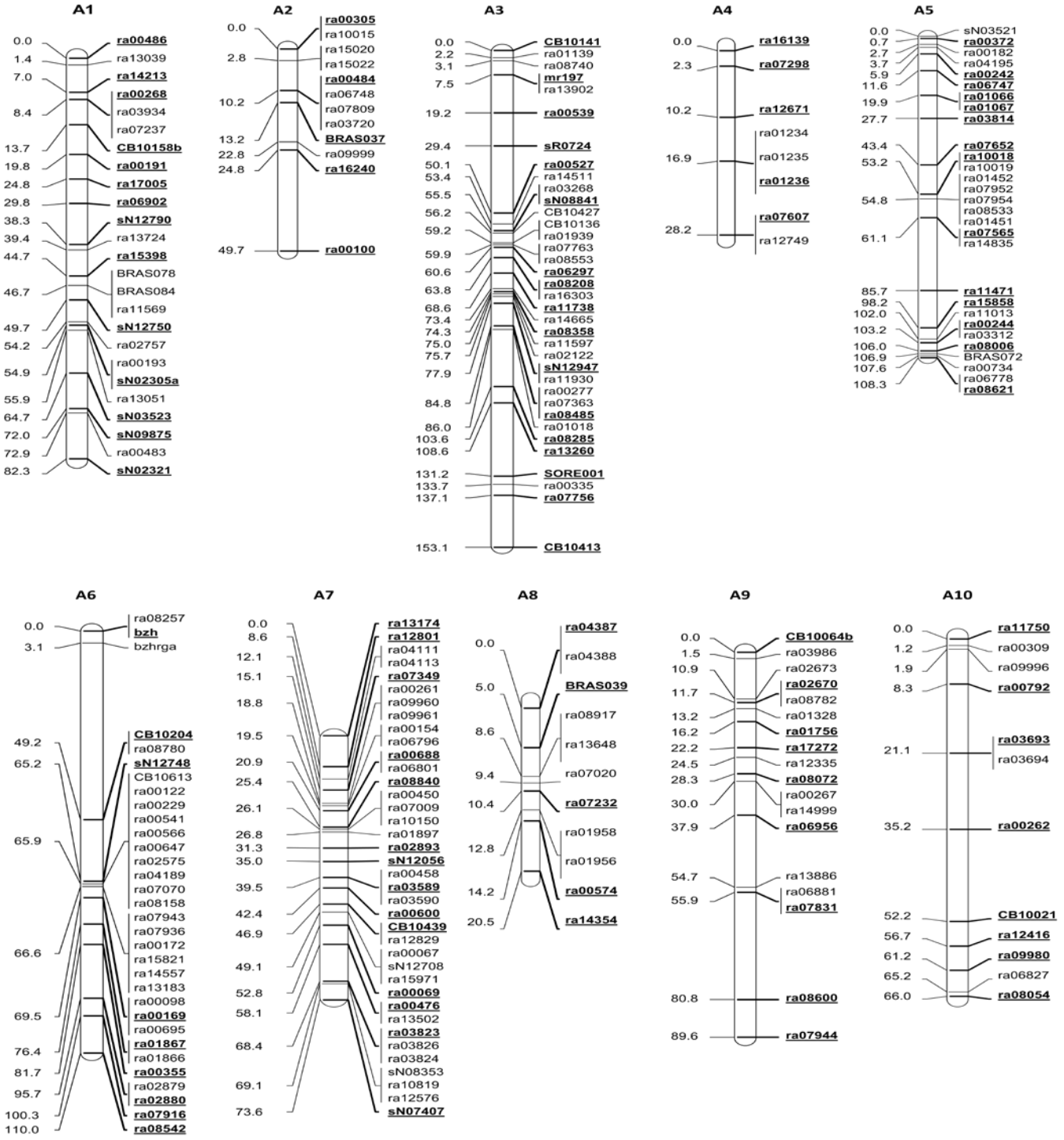
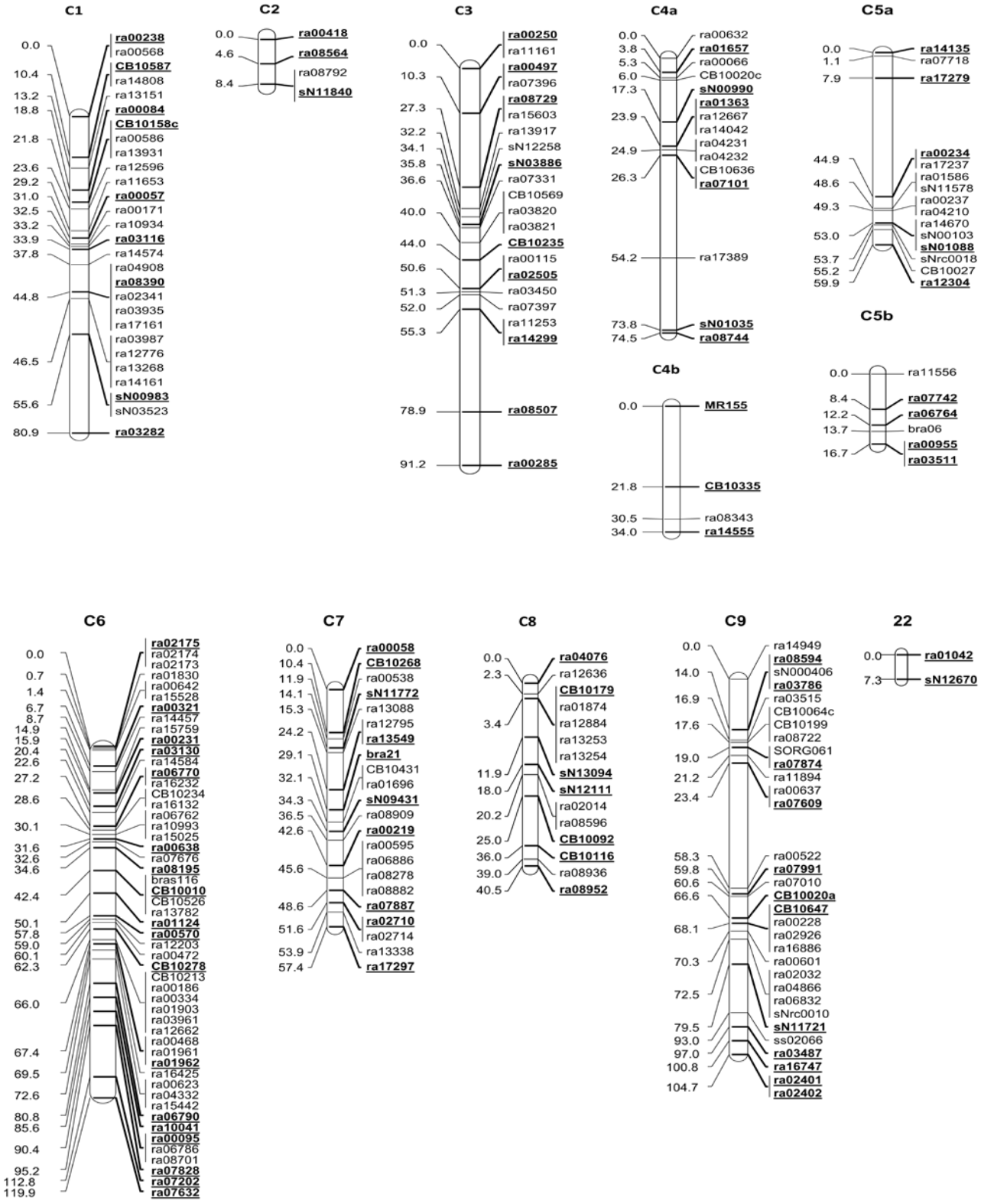


Figure 5: Linkage map of *Brassica napus* cross Alesi × H30. The vertical bars represent linkage groups N1-N10 = A1-A10 and N11-N19 = C1-C9 (international nomenclature). Marker locus names and positions (cM) are located to the left and right of the *vertical bars*, respectively.

Linkage map of Alesi × H30 Continued from page 168



Appendix 3

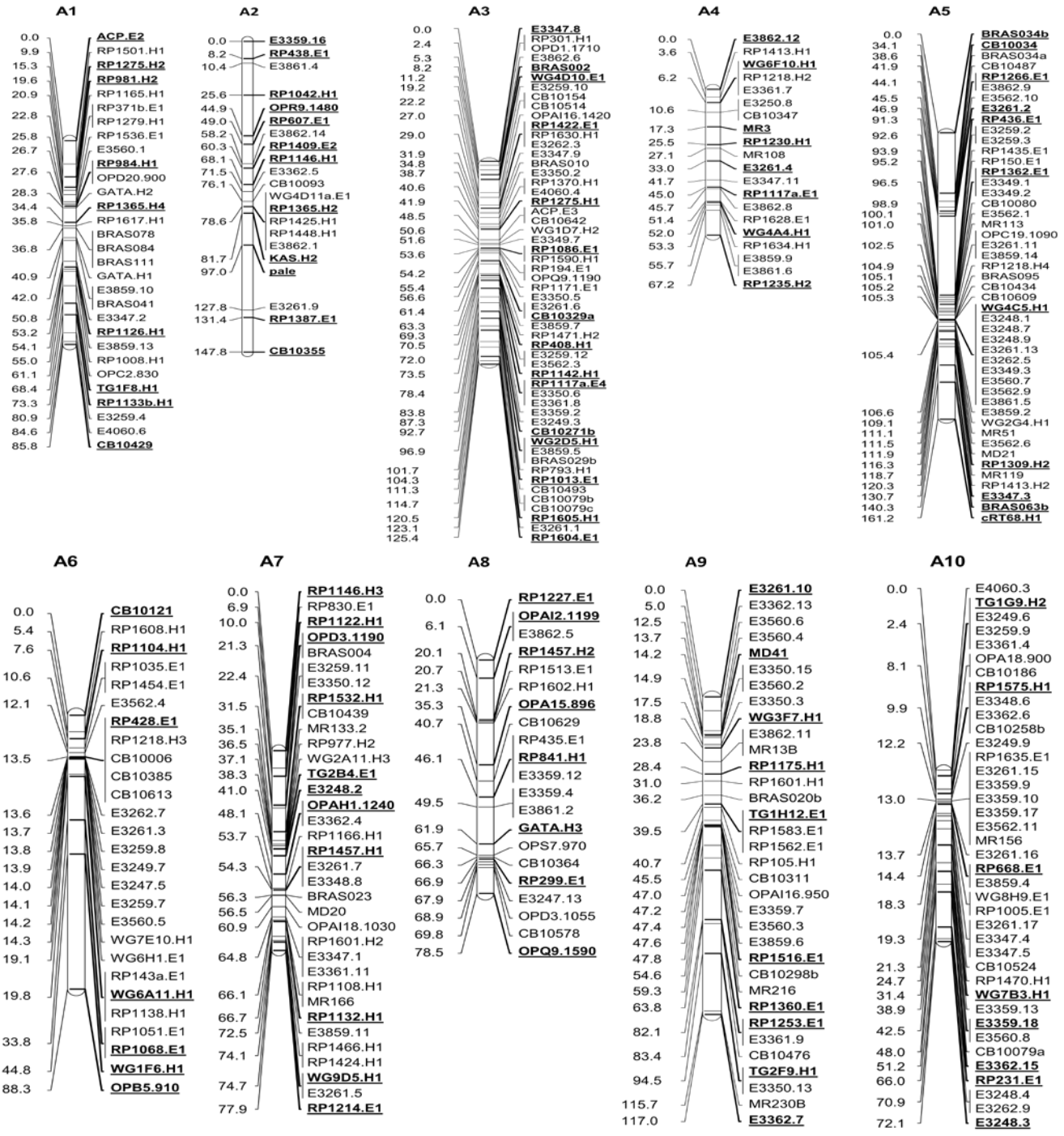
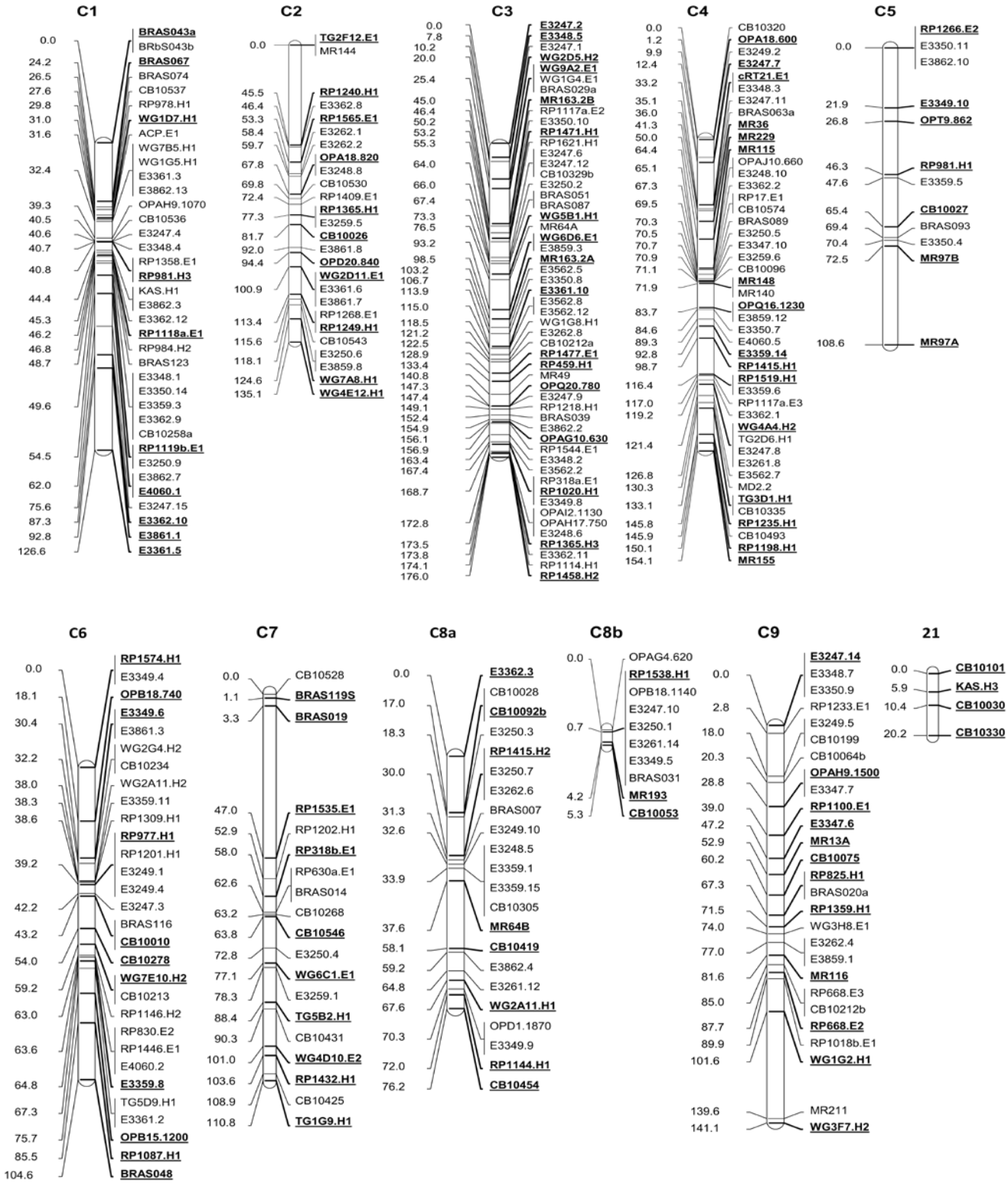
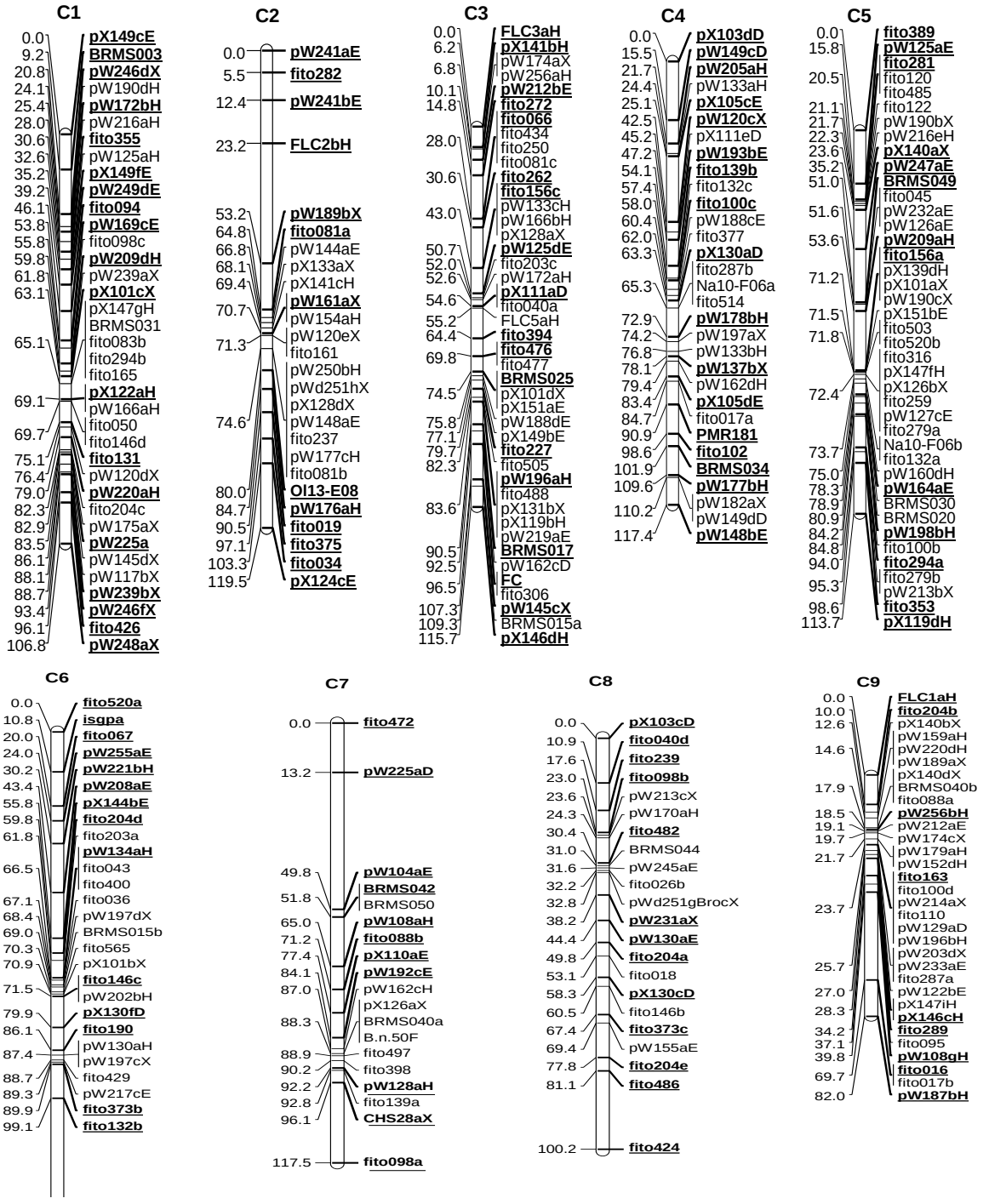


Figure 5: Linkage map of *Brassica napus* cross of Mansholts× Samourai based on a population of double haploid lines. *Vertical bars* represent linkage groups N1-N10 = A1-A10 and N11-N19 = C1-C9 (international nomenclature). Marker locus names and positions (cM) are located to the left and right of the *vertical bars*, respectively.

Linkage map of Mansholts × Samourai continued from page 170



Appendix 4



Linkage map of *Brassica oleracea* Bo1TBDH population. The vertical bars represent linkage groups N11-N19 = C1-C9 (international nomenclature). Marker locus names and positions (cM) are located to the left and right of the vertical bars, respectively. The markers that have been used for framework map construction are marked bold and underlined

Appendix 5

Salinity tolerant cultivars and lines of Brassica species developed through breeding

Brassica species	Cultivars/lines	Parameter for testing tolerance	References
B. napus	Dunkeld (canola)	Biomass and seed yield	Qasim (2000)
	ST9194	Germination	Puppala *et al.* (1999)
	Rapora, Mytnitskii, Chisayanatane	Seed yield	Pokrovskii (1990)
B. juncea	Common Green	Vegetative stage	Kwon *et al.* (1997)
	Varuna	Germination	Rai (1977)
		Seed yield	Kumar and Malik (1983), Kumar (1984)
	TH 68	Germination	Singh *et al.* (1984)
	RH 30	Seed yield	Dhawan *et al.* (1987), Kumar (1984)
	Pusa Bold, Kranti	Seed yield	Kumar (1995)
	CS4, CS15	Seed yield	Uma *et al.* (1992)
	Pant Rai 2030	Seed yield	Sinha (1991)
	RH 7818	Seed yield	Dhawan *et al.* (1987)
	DIRA 337	Seed yield	Sinha (1991)
	BM-1, LL-84	Biomass and seed yield	Ashraf (1992)
	P-15, KS-51	Biomass and seed yield	Ashraf *et al.* (1994)
B. carinata	C90-1191, P5/80, Yellow Dodella	Germination and seedling growth	Ashraf and Sharif (1997)
	C90-1115, 77-321	Seed yield	Ashraf and Sharif (1998)
B. rapa	BSH1	Germination	Paliwal (1972)
		Seed yield	Kumar (1984)

- For references listed in this table see (Nayidu et al. 2013)

Appendix 6

Minimum, maximum and of fresh weight (FW), dry weight (DW), relative water content (RWC), chlorophyll content measured by SPAD (SPAD1, SPAD2), Sodium content (Na^+ mg/g D.M.), Potassium content (K^+ mg/g D.M.) of *Brassica napus* DH population (Mansholts × Samourai) (n =138), and parental lines under control and salt stress (200 mM NaCl).

Traits	DH population						Mansholts		Samourai	
	Min		Max		Mean		Mean		Mean	
	Control	Salt	Control	Salt	Control	Salt	Control	Salt	Control	Salt
FW(g)	2.09	1.58	7.32	4.15	4.58	2.67	5.2	2.91	5.1	2.79
DW(g)	0.34	0.22	0.93	0.65	0.59	0.42	0.68	0.48	0.63	0.48
RWC	82.83	82.45	91.57	87.64	87.19	84.62	87.70	83.58	87.06	82.80
SPAD1	19.88	35.78	43.93	48.57	38.06	42.31	42.99	46.25	39.91	44.83
SPAD2	19.93	36.01	44.79	52.81	38.87	44.89	42.47	46.82	39.60	46.16
Na^+ mg/g DM	0.76	14.57	2.40	35.92	1.14	24.16	1.05	21.65	1.10	24.43
K^+ mg/g DM	34.02	30.06	81.03	63.27	47.95	50.29	49.64	46.57	49.82	46.20
Na^+/K^+	0.02	0.27	0.25	0.76	0.03	0.48	0.020	0.46	0.021	0.53

Min = Minimum, Max = maximum

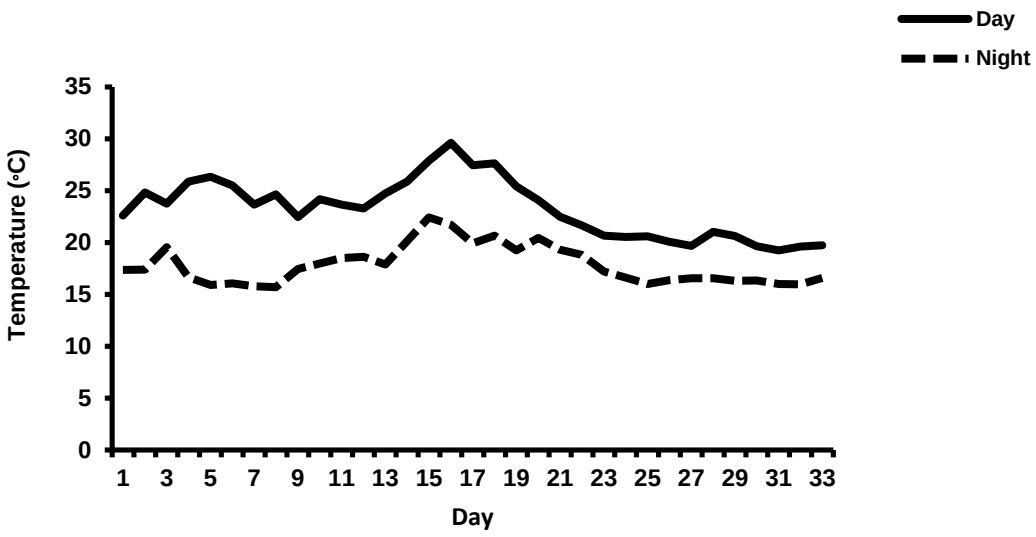
Appendix 7

Minimum, maximum and of fresh weight (FW), dry weight (DW), relative water content (RWC), chlorophyll content measured by SPAD (SPAD1, SPAD2), Sodium content (Na^+ mg/g D.M.), Potassium content (K^+ mg/g D M) of *Brassica oleracea* DH population Bo1TBDH (n =138), and parental lines under control and salt stress (100 mM NaCl).

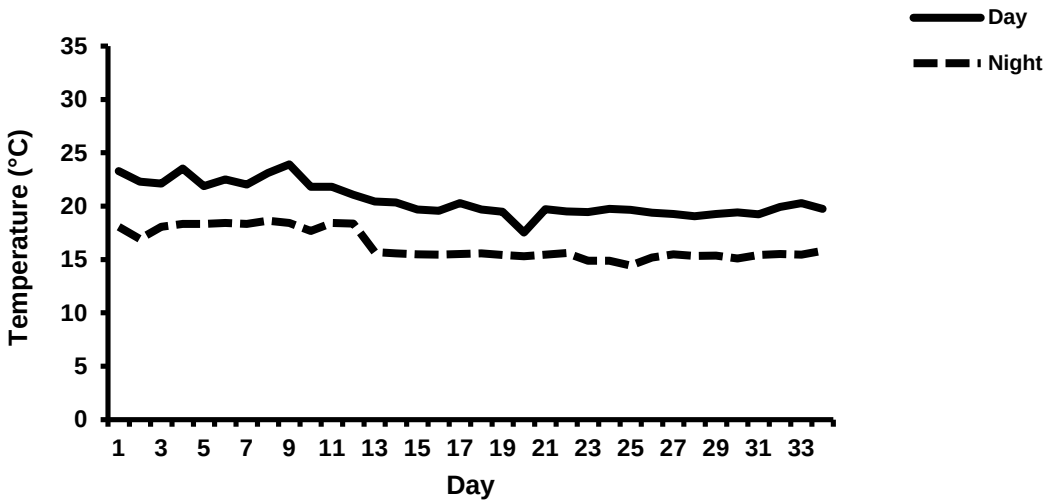
Traits	DH population						TO1000DH3		Early Big	
	Min		Max		Mean		Mean		Mean	
	Control	Salt	Control	Salt	Control	Salt	Control	Salt	Control	Salt
FW(g)	2.27	0.96	6.18	3.97	4.14	2.84	3.42	2.05	3.89	2.4
DW(g)	0.11	0.14	0.58	0.57	0.42	0.35	0.33	0.20	0.39	0.28
RWC	86.72	42.93	95.30	93.77	89.75	87.18	88.89	86.77	89.72	87.24
SPAD	34.43	42.10	62.20	66.84	52.25	55.12	48.82	49.54	53.8	54.32
Na^+ mg/g DM	1.43	17.51	4.44	54.39	2.73	28.17	2.68	26.90	2.60	21.76
K^+ mg/g DM	57.67	22.30	81.61	59.61	67.94	44.59	67.13	51.77	58.97	50.37
Na^+/K^+	0.02	0.27	0.07	0.76	0.04	0.65	0.04	0.36	0.04	0.32

Min = Minimum, Max = maximum

Appendix 8



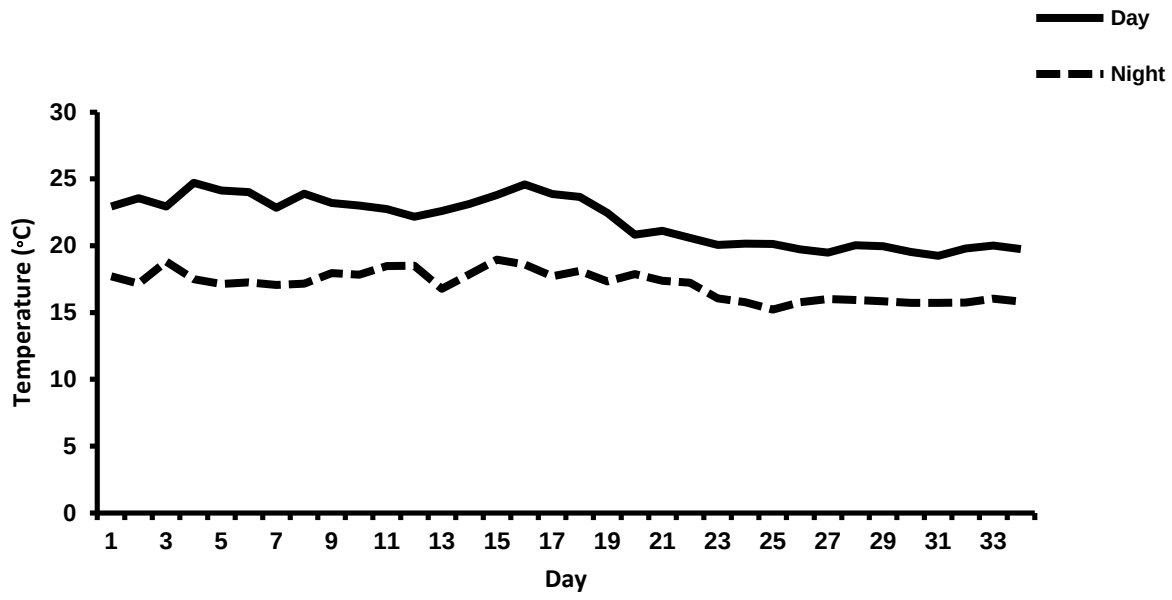
Temperature of replicate one day and night



Temperature of replicate two day and night

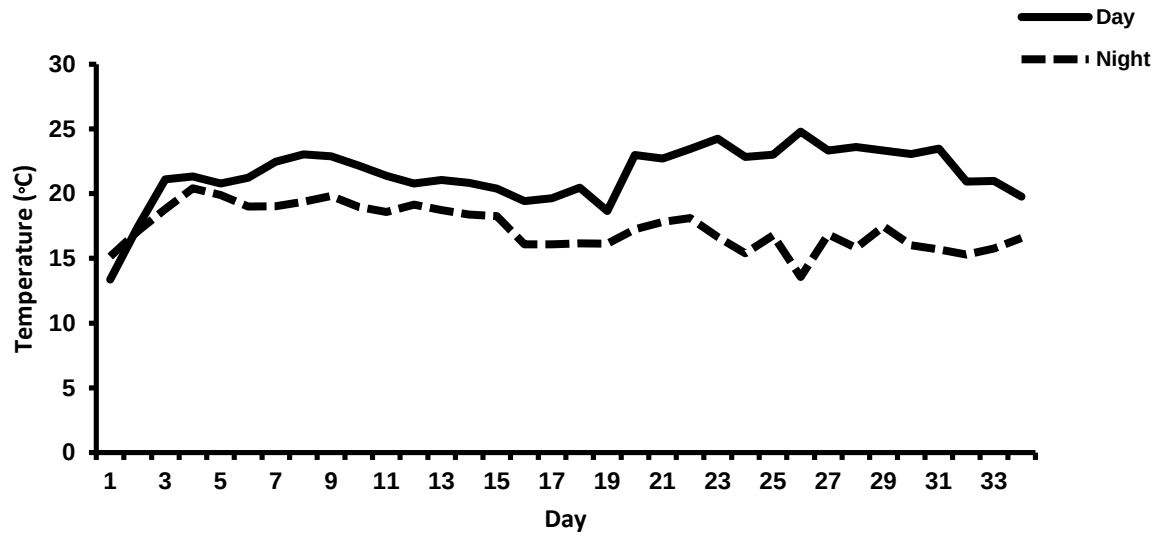
The fluctuation of temperature fluctuation day and night across the duration of the experiment for *B. napus* DH population Mansholts × Samourai for replicate one and replicate is presented for both replicates

Appendix 8 continued from page 176



Average of temperature overall the two replicates

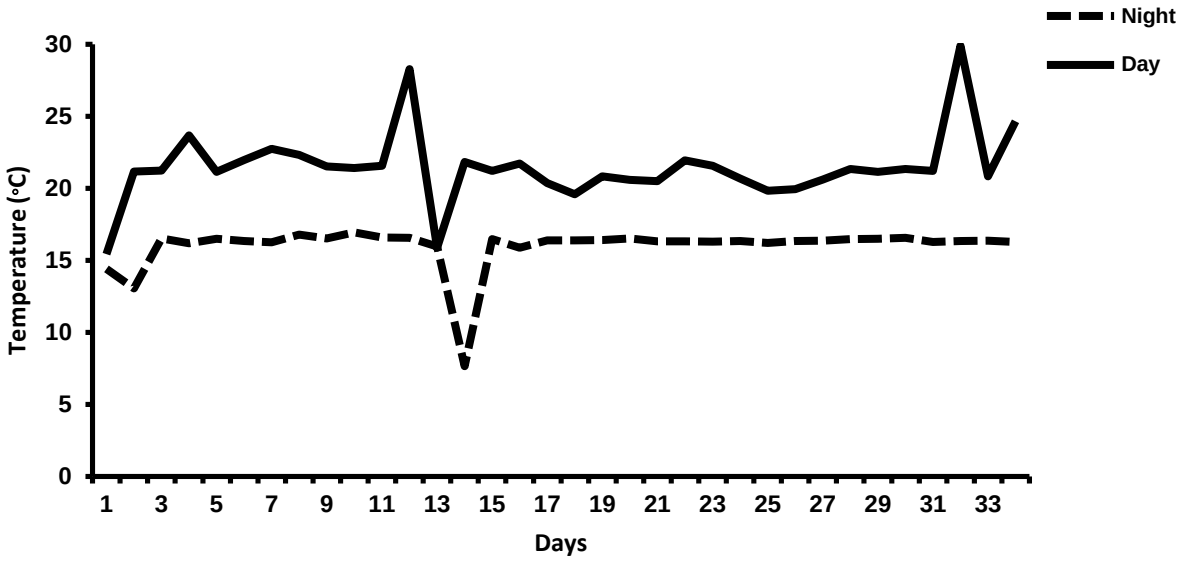
Appendix 9



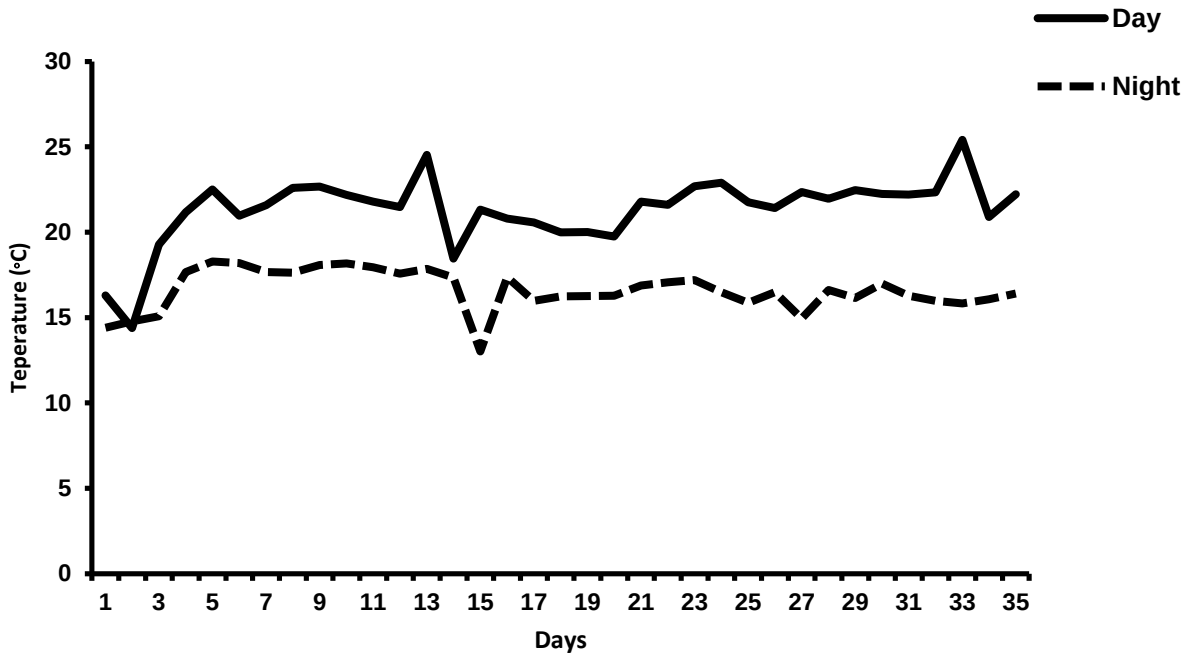
Temperature of replicate one

The fluctuation of temperature fluctuation day and night across the duration of the experiment for *B. oleracea* Bo1TBDH DH population for replicate one and replicate is presented for both replicates.

Appendix 9 continued from page 177



Temperature of replicate two



Temperature mean overall the two replicates

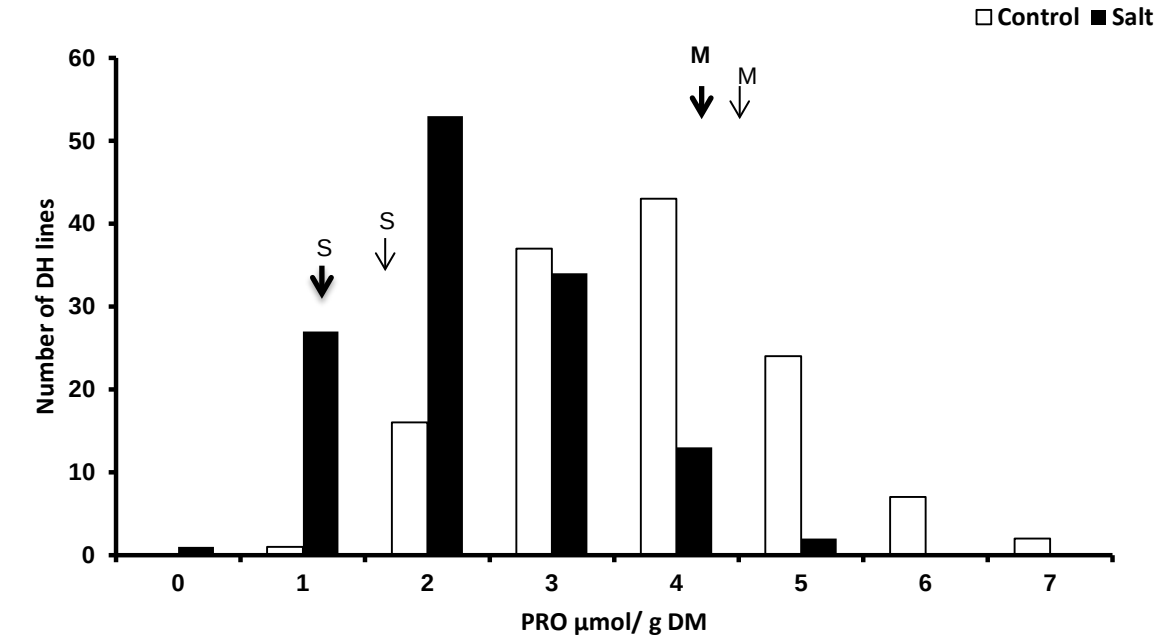
Appendix 10

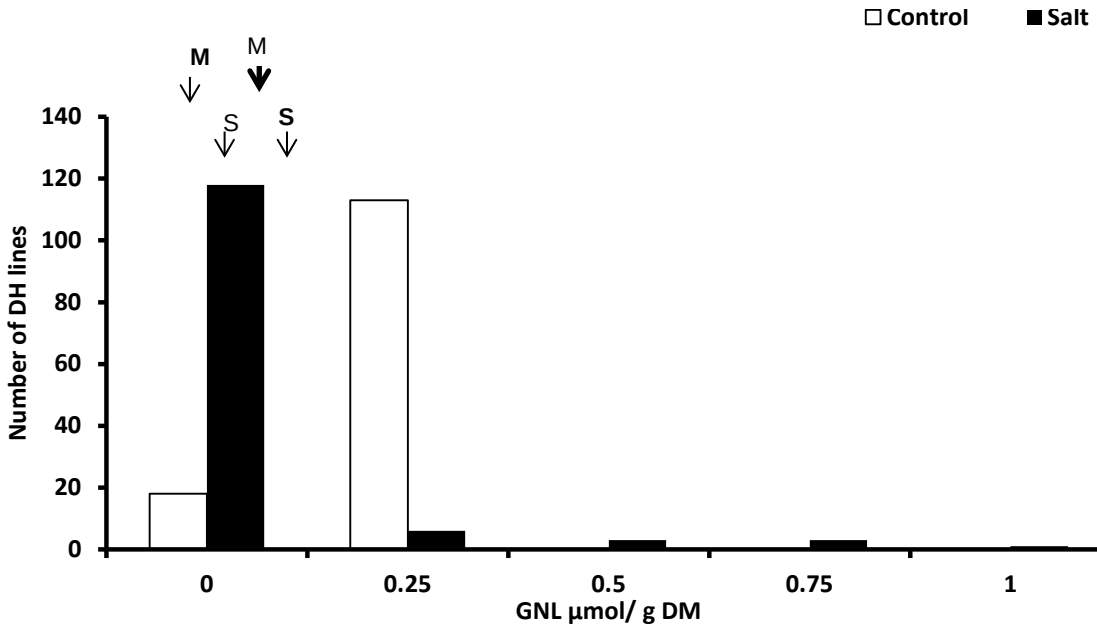
Mean squares and F test of significance from the ANOVA and heritabilities of glucosinolates for *B.napus* DH population Mansholts × Samourai, n = 138 DH lines under control condition and salt stress (200 mM NaCl).

	Control				Salt			
Sources of Variance	Genotypes (G)	Replicates (R)	G ×R	h^2	Genotypes (G)	Replicates (R)	G ×R	h^2
DF	137	1	137		137	1	137	
PRO	3.30**	8.84	1.1	66.68	1.82**	0.39**	0.45	75.49
GNL	0.005	0.0005	0.005	7.77	0.03**	0.02**	0.004	87.41
RAA	0.03**	0.204**	0.019	31.37	0.13**	0.39*	0.08	40.11
RAE	0.10+	2.45**	0.09	20.37	0.15**	1.57**	0.01	35.88
GNA	0.9,**	13.41**	0.28	70.00	0.13**	0.50**	0.06	54.26
4OH	0.2**	0.39**	0.1	44.89	0.006**	0.003	0.003	44.95
GBN	1.1**	0.48	0.58	47.51	1.13**	6.09**	0.43	61.73
GBC	0.74	59.30**	0.55	14.94	2.08**	0.89	0.58	71.87
NAS	0.17+	6.74**	0.13	22.43	0.04**	0.86**	0.02	59.11
4 ME	0.0063+	0.14**	0.005	22.86	0.011**	0.04**	0.002	79.01
NEO	0.02	0.02	0.02	4.73	0.64**	0.18	0.15	75.97
SUM	18.52**	3.59	5.96	67.96	9.86**	7.78*	2.12	78.51

** Significant at *P*=0.01; * significant at *P*=0.05 and + significant at *P*=0.1

Appendix 11

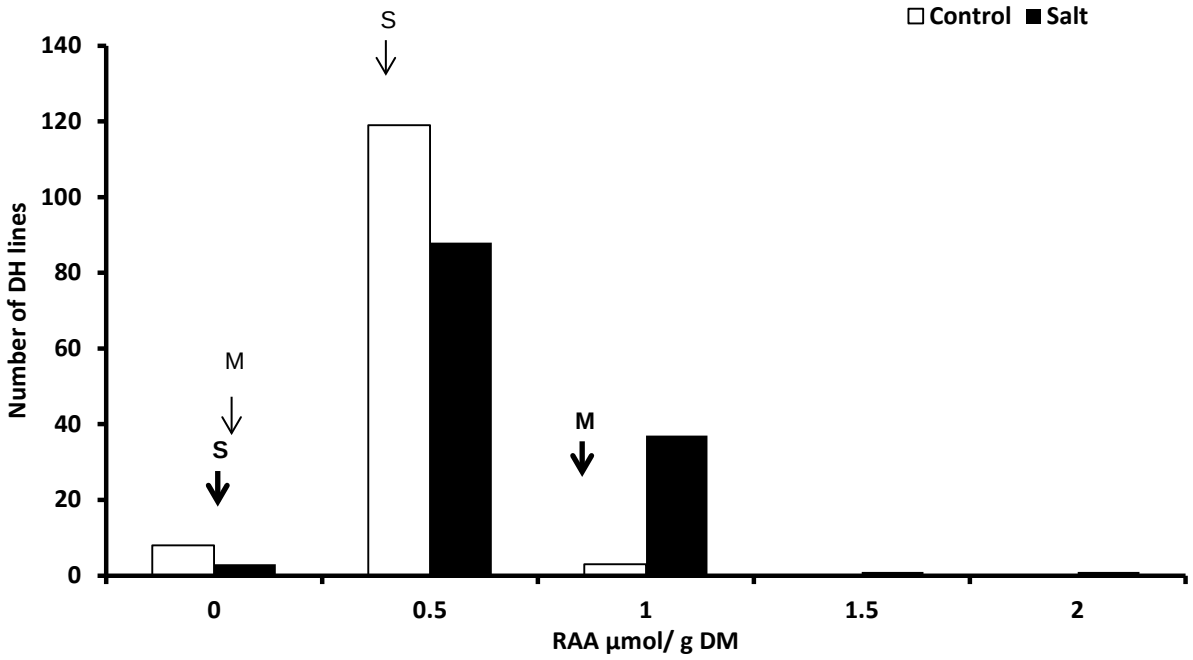


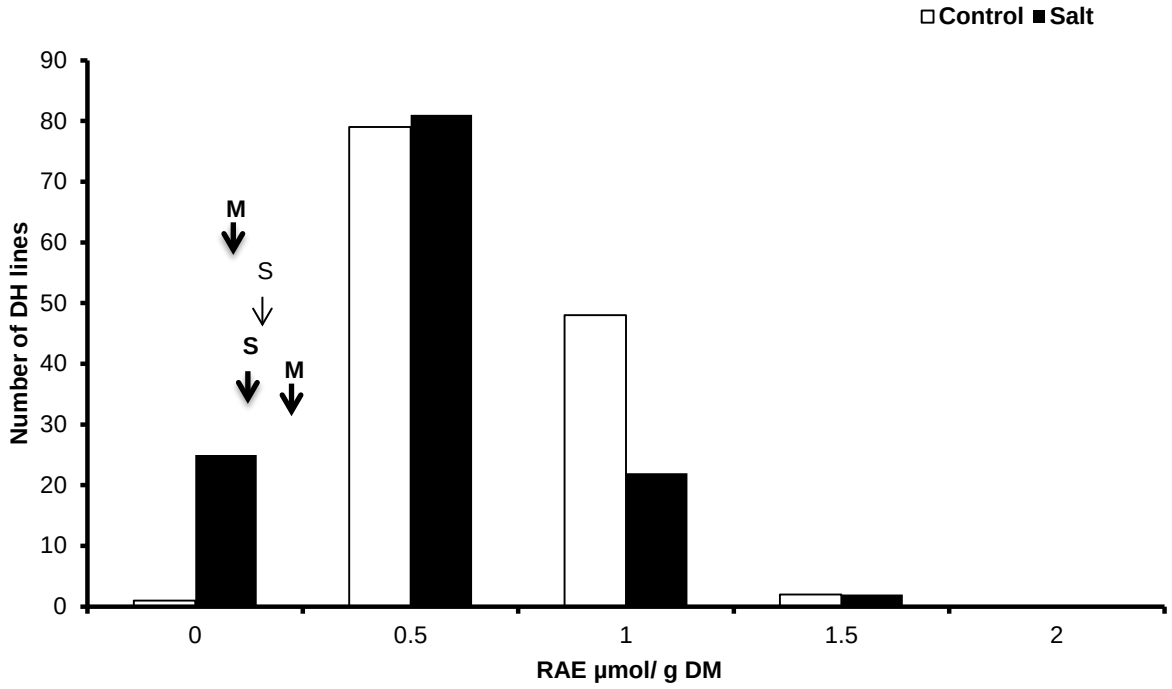


Distribution of single components and total glucosinolates under control and salt stress in B. napus DH population Mansholts × Samourai, n = 138 DH lines under control condition and salt stress (200 mM NaCl).

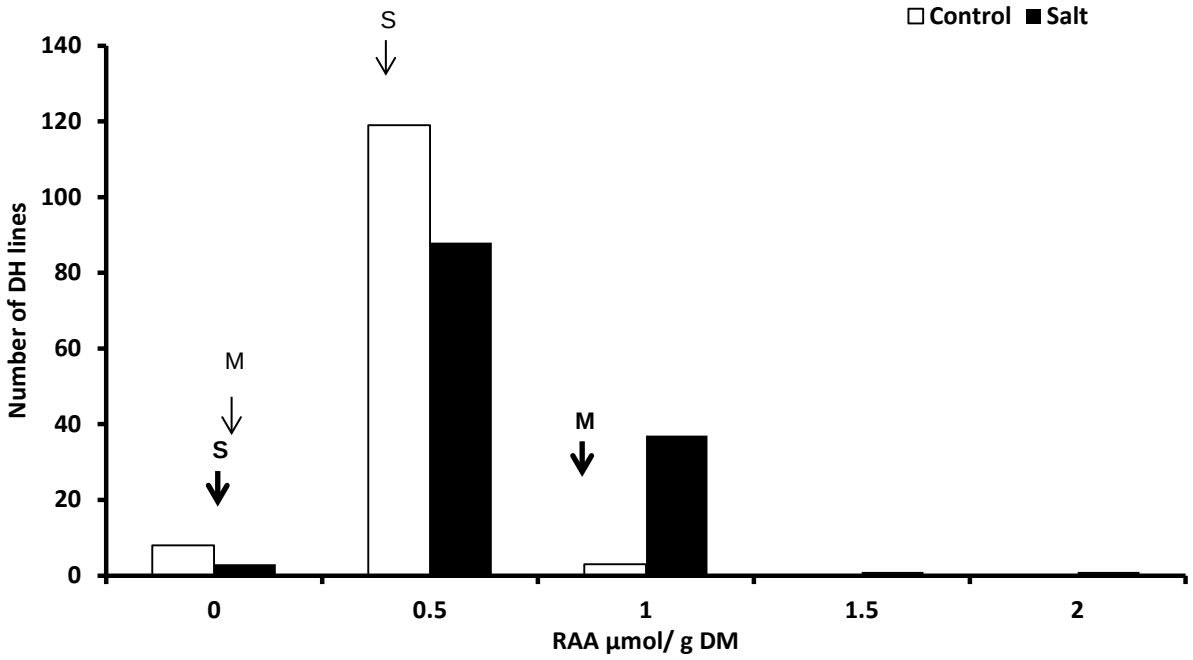
M = Mansholts, S = Samourai, regular format arrows = parents performance under control and **Bold format arrows** = parents performance under salt stress.

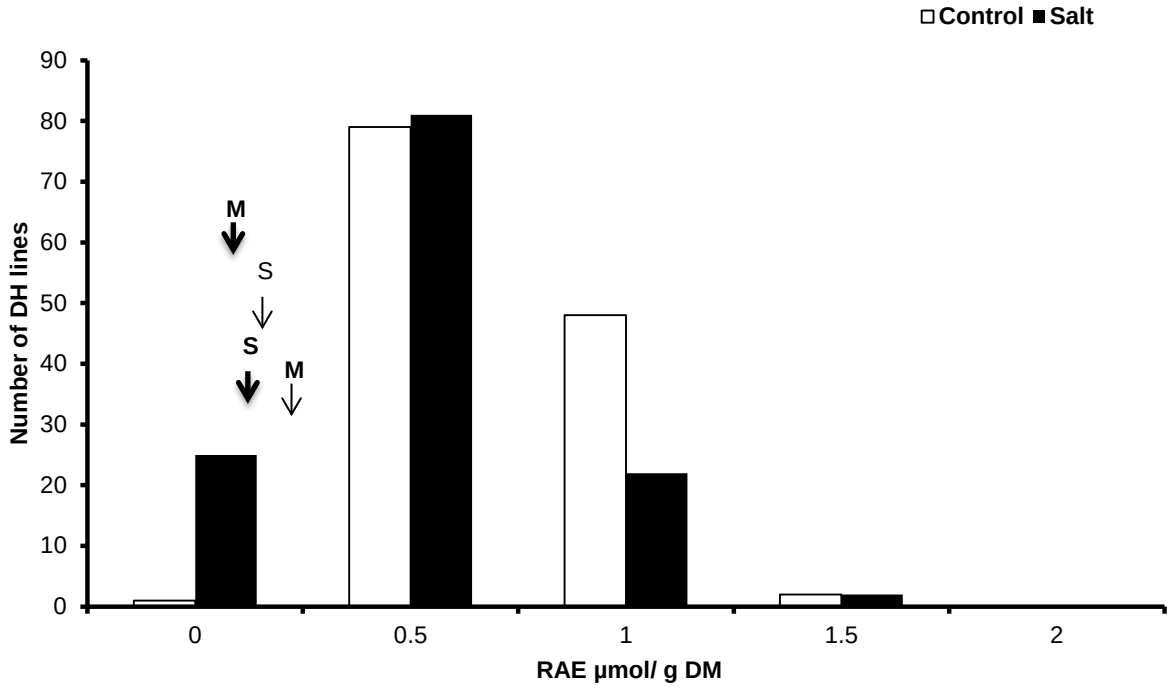
Appendix 11 continued from page 180



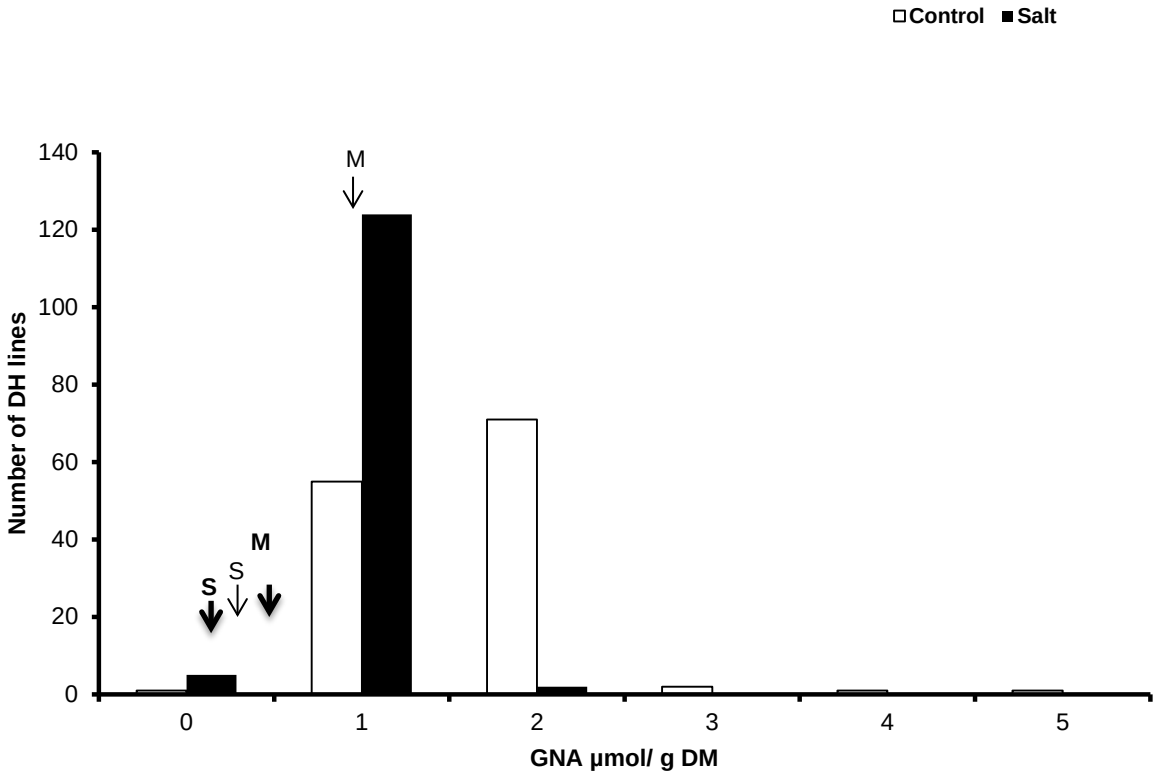


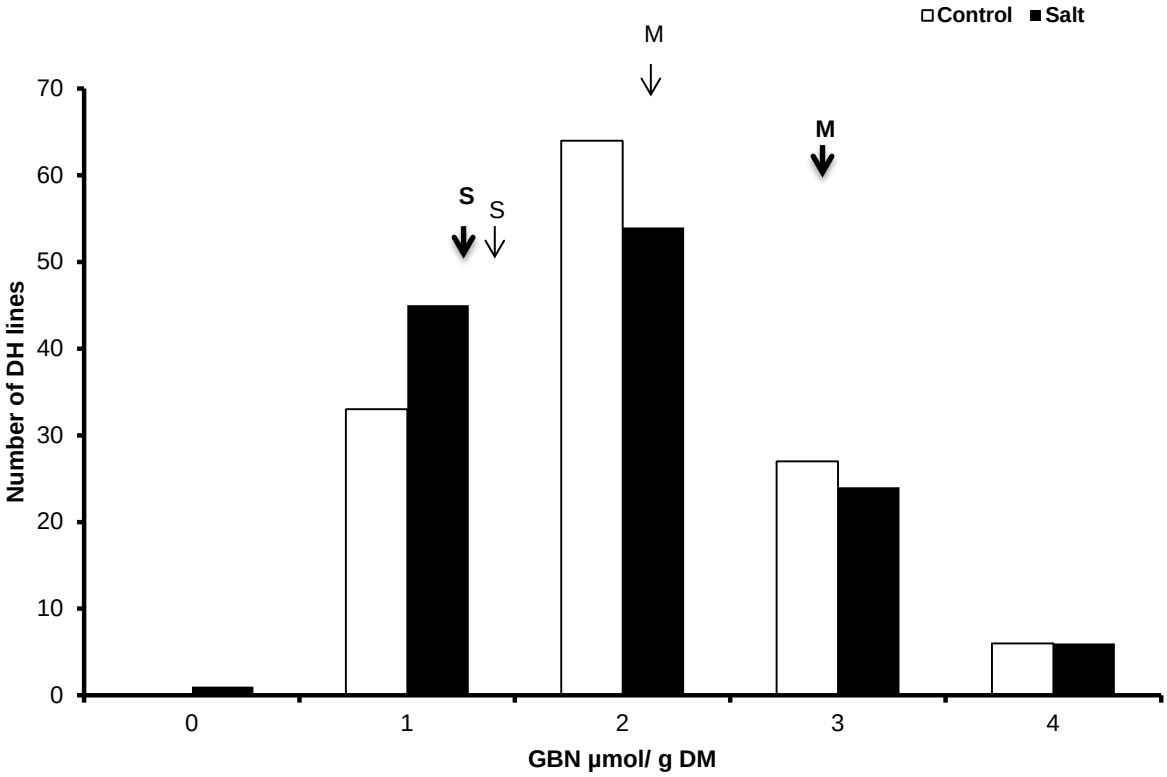
Appendix 11 continued from page 181





Appendix 11 continued from page 182





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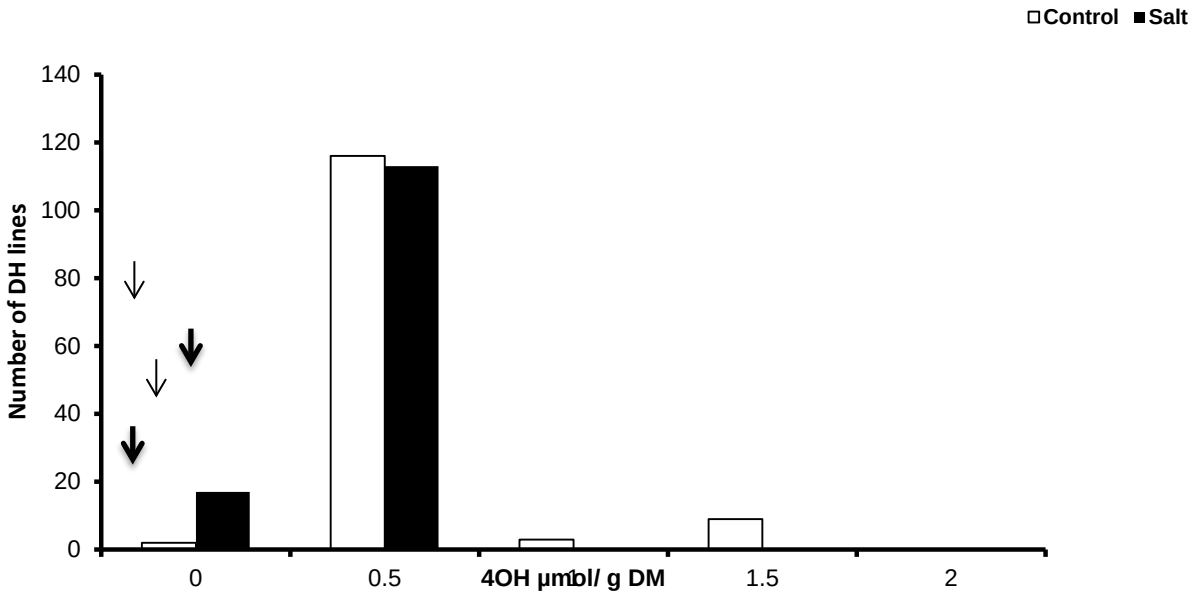
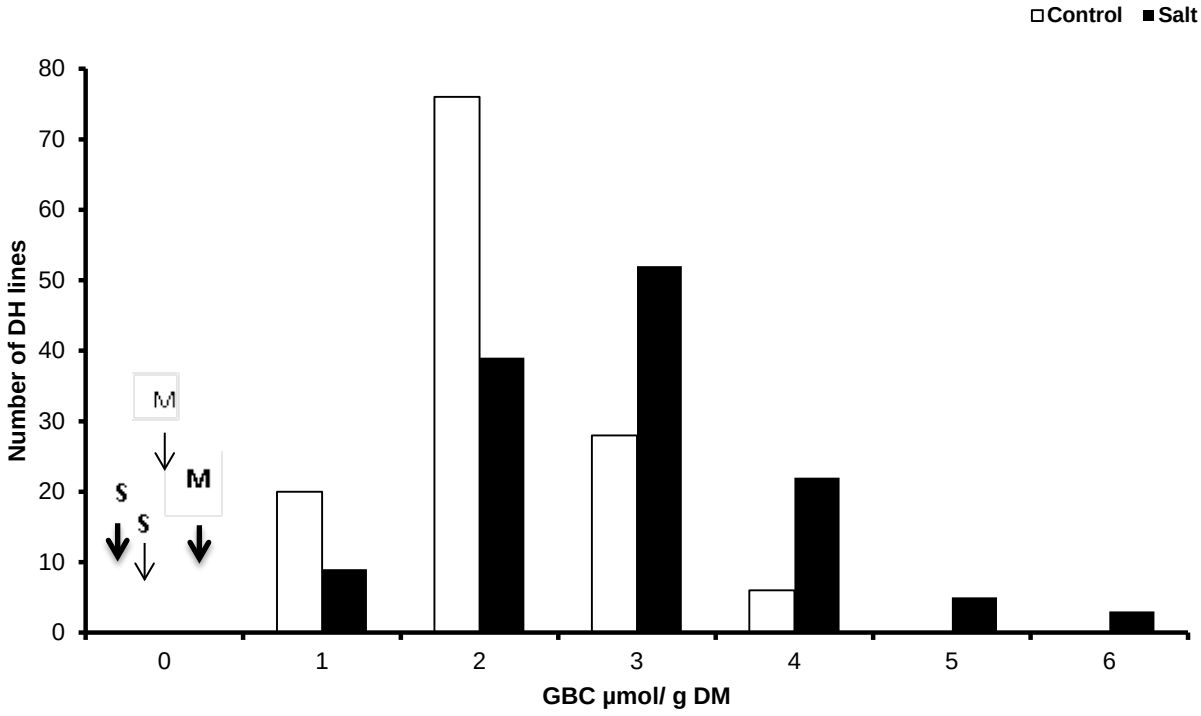
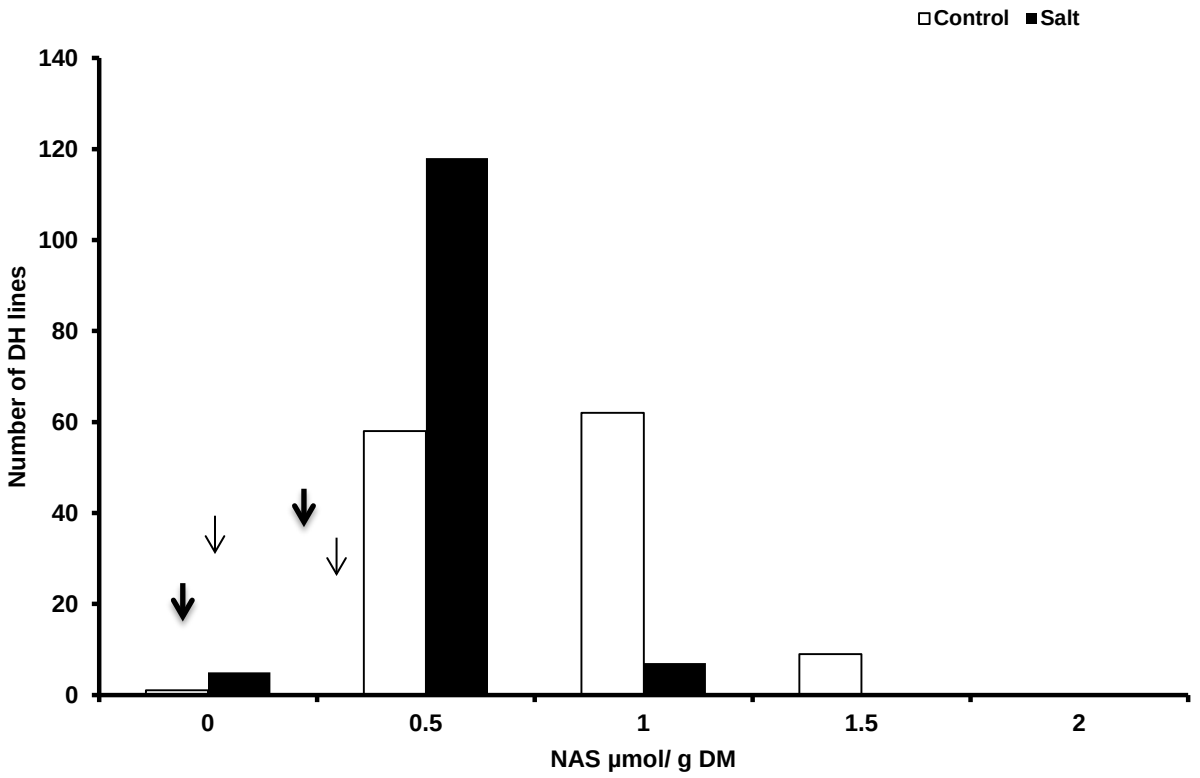
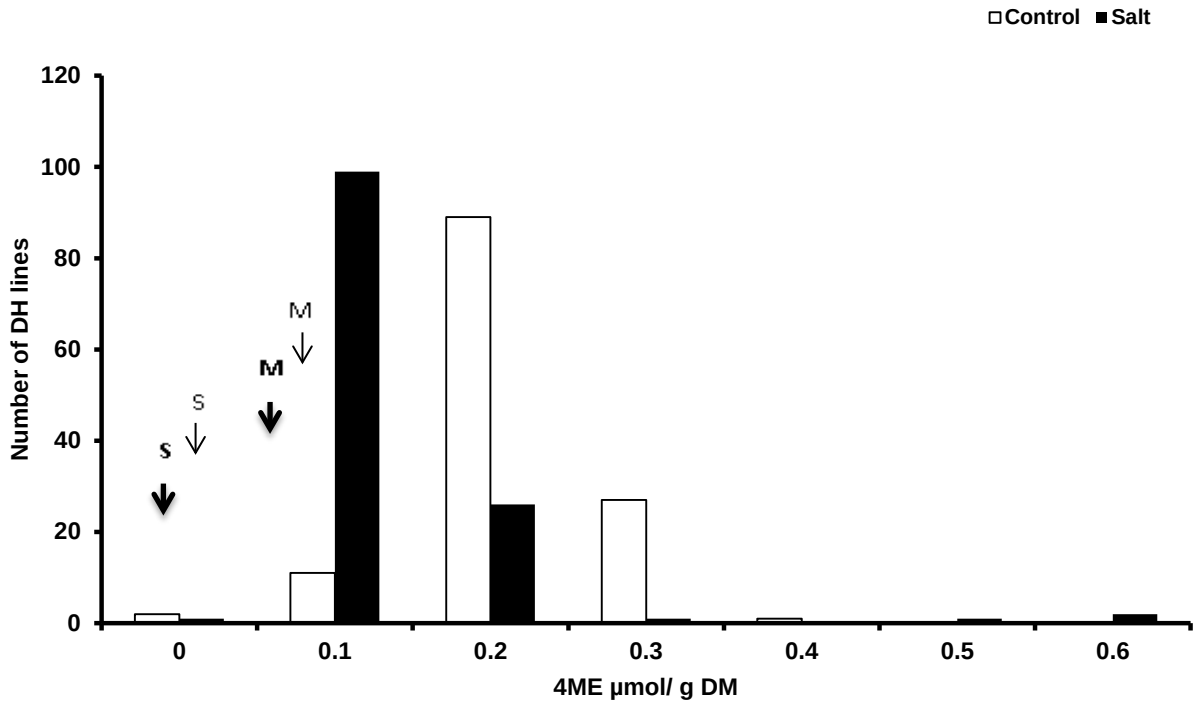


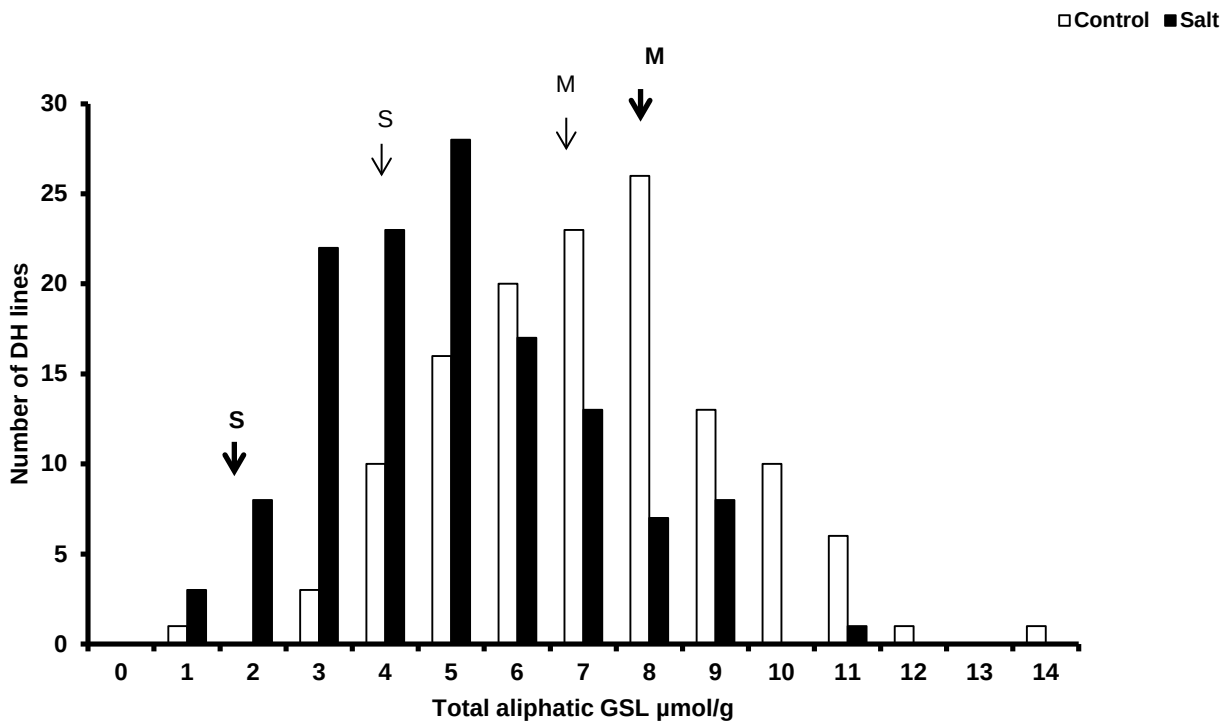


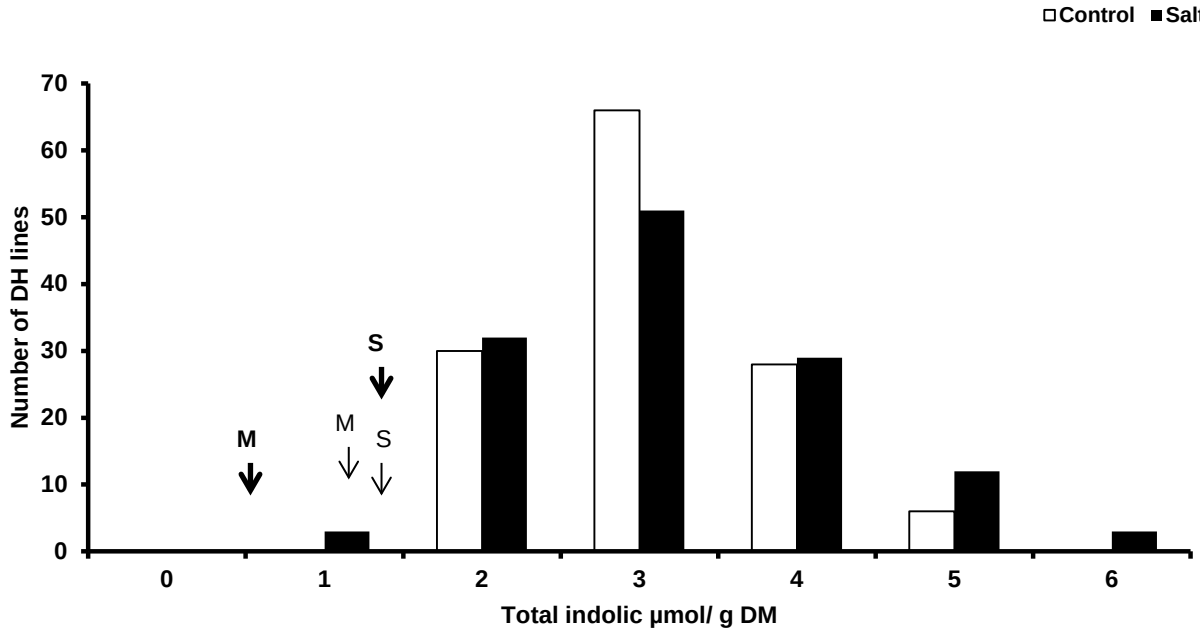
Appendix 11 continued from page 184





Appendix 11 continued from page 185





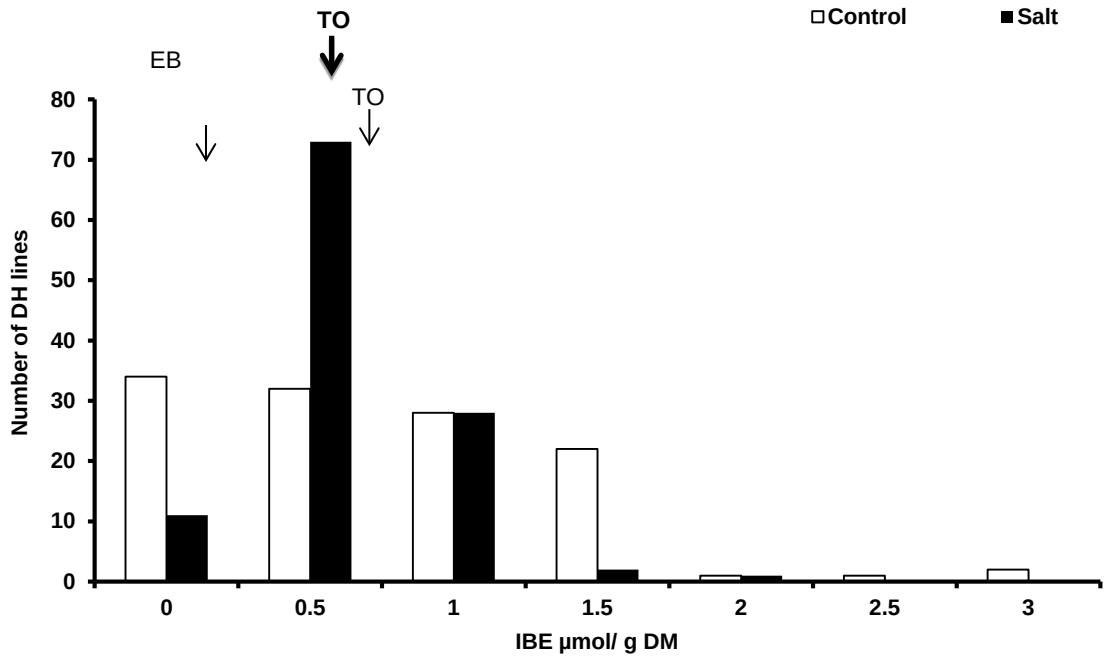
Appendix 12

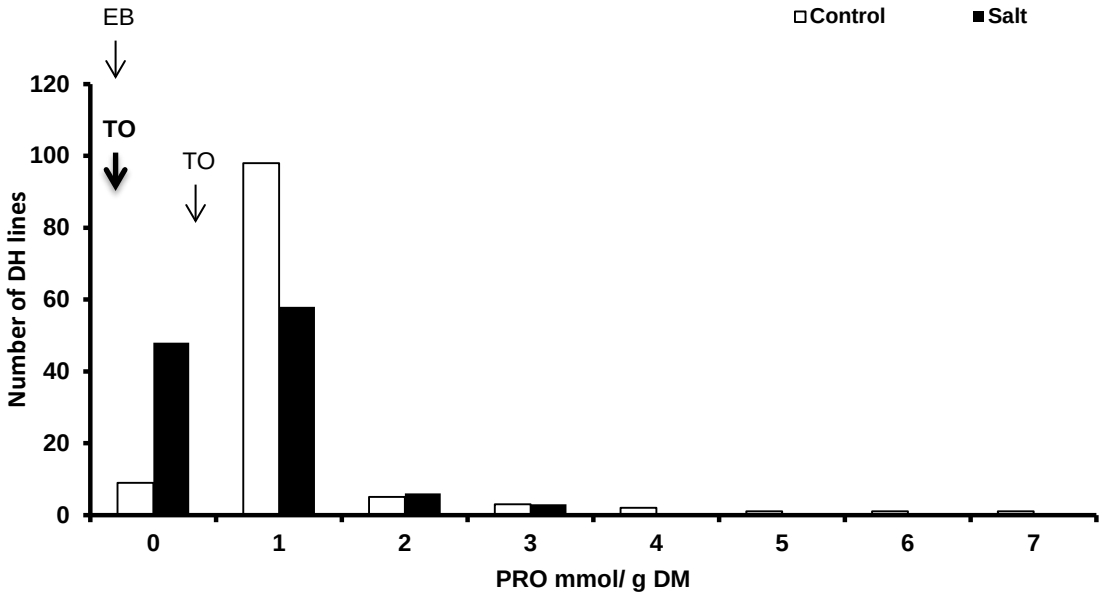
Mean squares and F test of significance from the ANOVA and heritabilities of glucosinolates for *B. oleracea* DH population Bo1TBDH, n = 138 DH lines under control condition and salt stress (100 mM NaCl).

	Control				Salt			
Sources of Variance	Genotypes (G)	Replicates (R)	G ×R	h^2	Genotypes (G)	Replicates (R)	G ×R	h^2
DF	137	1	137		137	1	137	
IBE	0.62**	1.84*	0.32	47.98	0.17	0.08	0.18	-
PRO	1.63**	0.250	0.21	87.02	0.45	0.05	0.52	-
GNL	0.40**	4.67**	0.25	36.75	0.44	0.24	0.45	-
RAA	0.002*	0.004+	0.001	27.76	0.001	0.001	0.001	-
GNA	5.83**	5.73*	0.88	84.81	2.78	0.15	3.31	-
4OH	0.002*	0.07**	0.001	31.04	0.0009	0.002	0.001	-
GBN	-	-	-	-	0.0004	0.000	0.001	-
GBC	4.88**	2.56	1.83	62.41	1.077	2..33	1.09	-
NAS	0.10	1.25**	0.08		0.035	0.01	0.04	-
4 ME	0.02**	0.25**	0.01	39.97	0.007	0.02	0.006	14.95
NEO	2.45	0.300	1.97	19.43	0.75	2.49	0.86	-
SUM	18.55**	5.46	8.47	54.30	5.99	22.40	6.98	-

** Significant at *P*=0.01; * significant at *P*=0.05 and + significant at *P*=0.1

Appendix 13

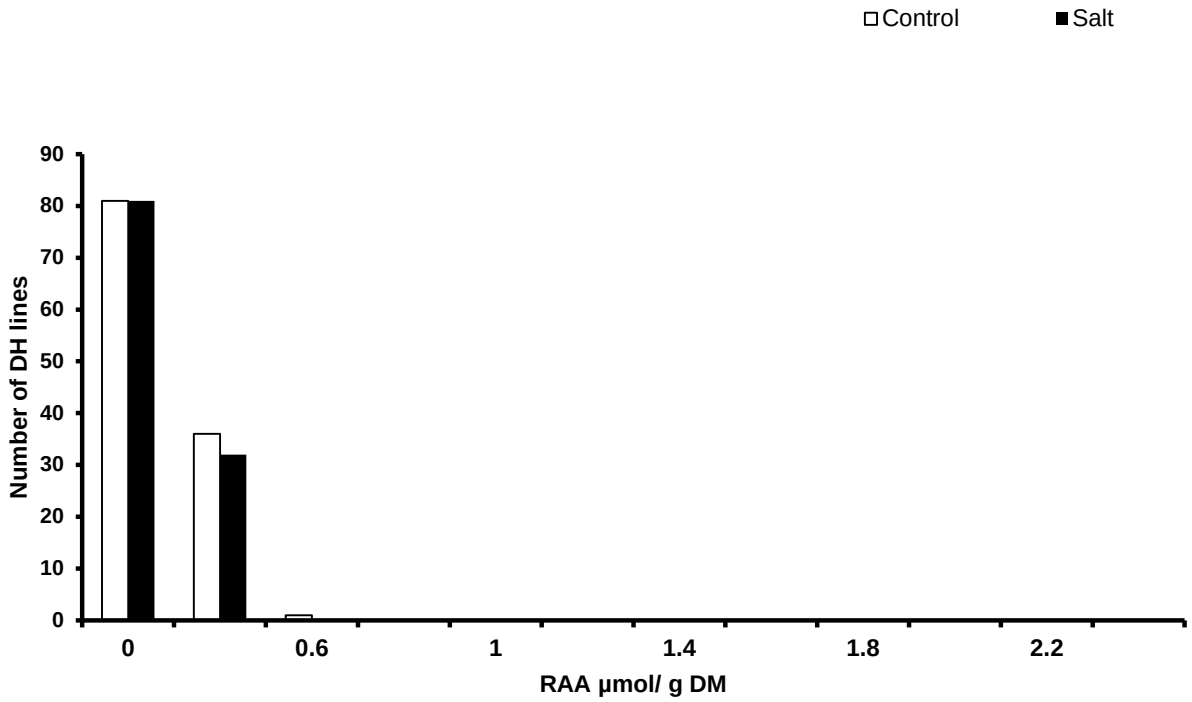




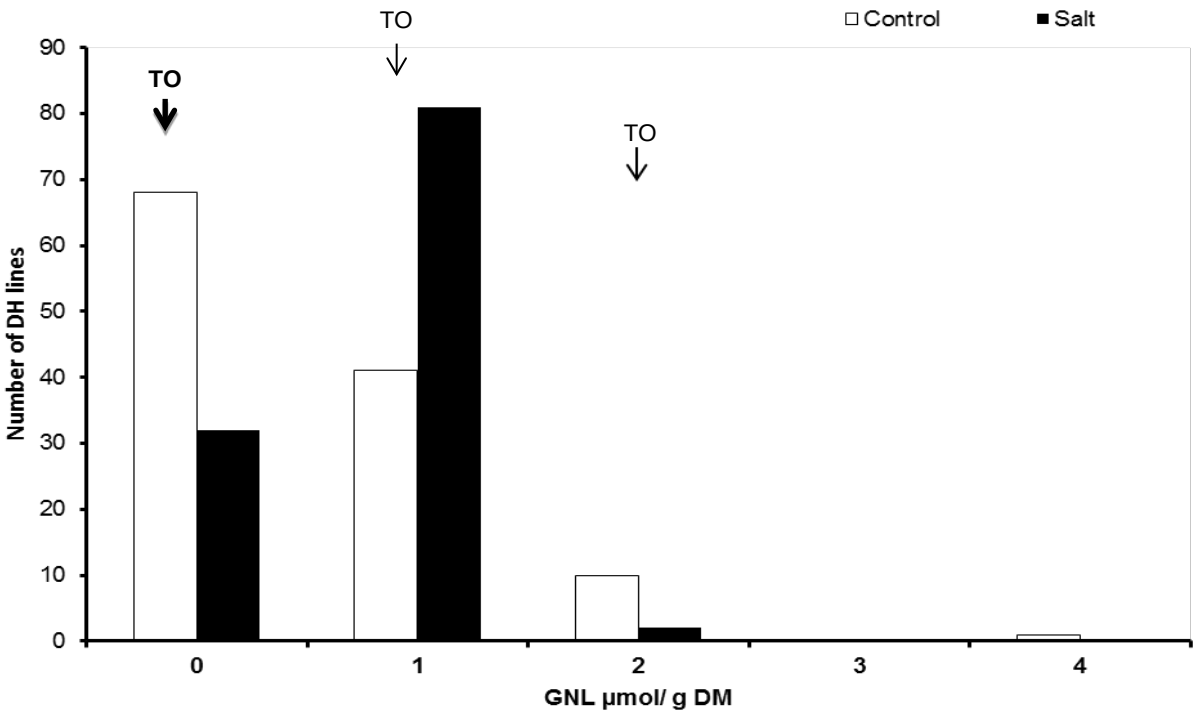
Distribution of glucosinolates for *B. oleracea* DH population Bo1TBDH, n = 138 DH lines under control condition and salt stress (100 mM NaCl).

TO = TO1000DH3, EB = Early Big, regular format arrows = parents performance under control and **Bold format arrows** = parents performance under salt stress

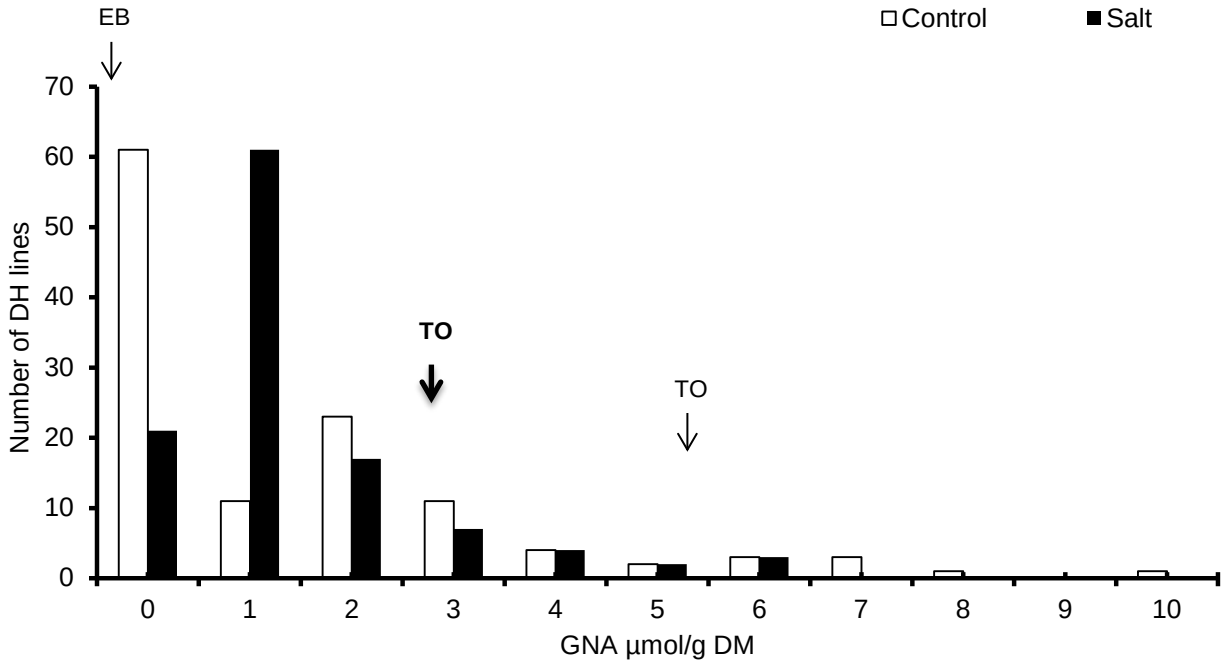
Appendix 13 continued from page 188

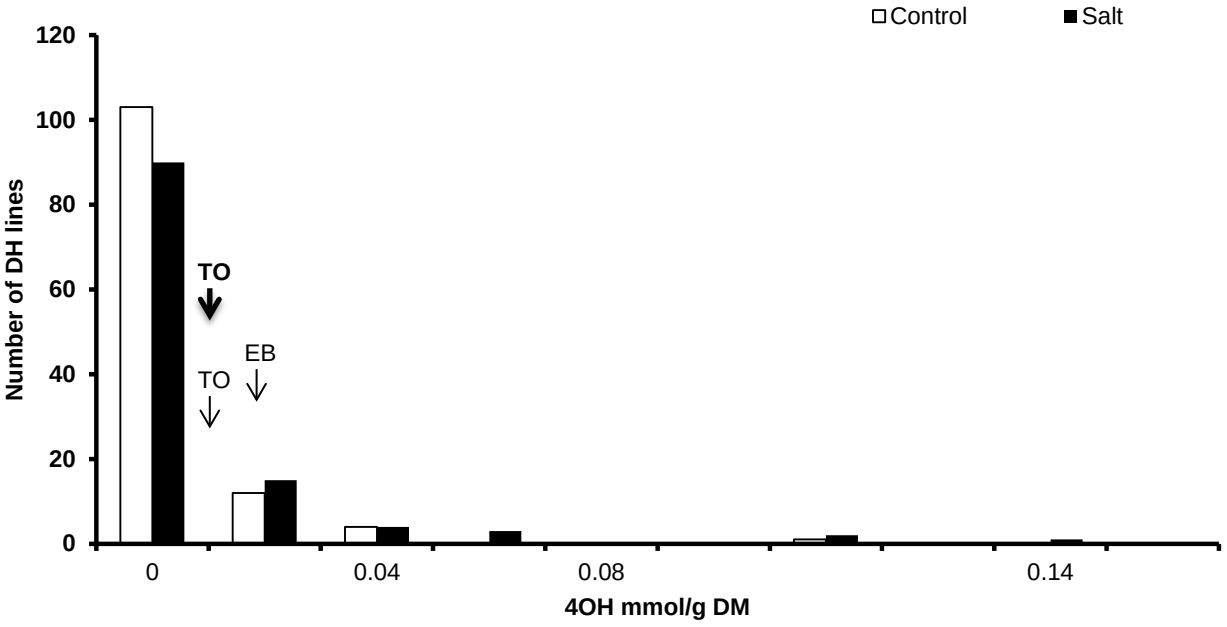


RAA was not detected in both parents

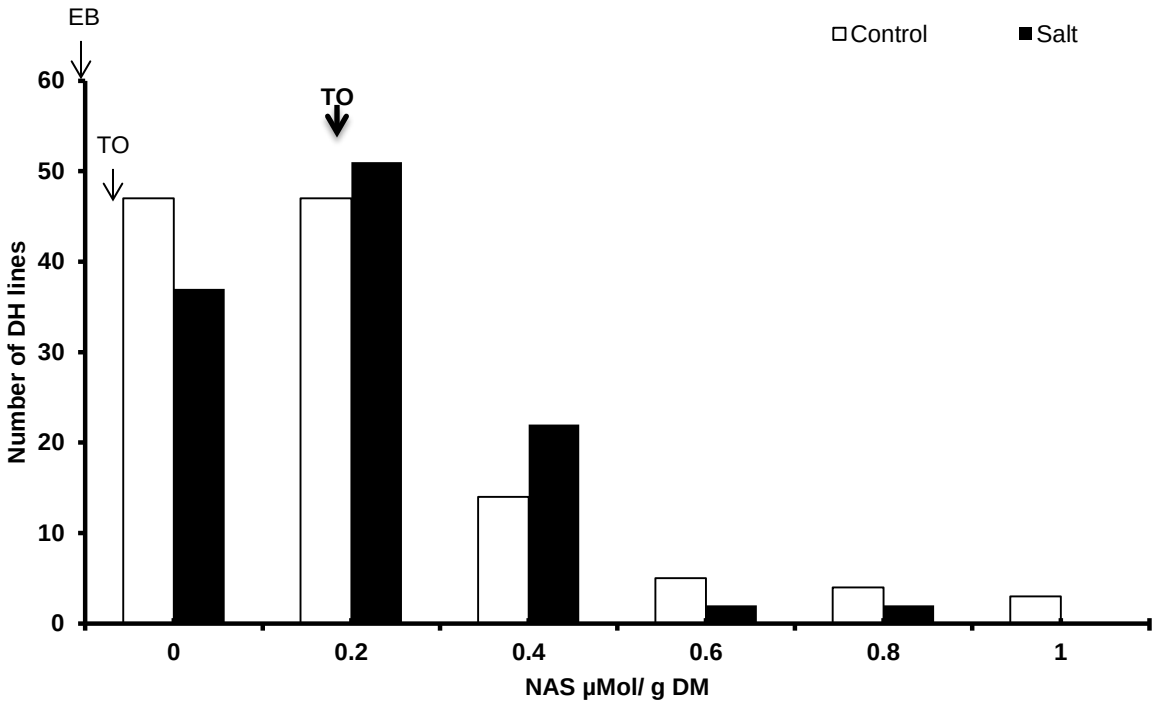


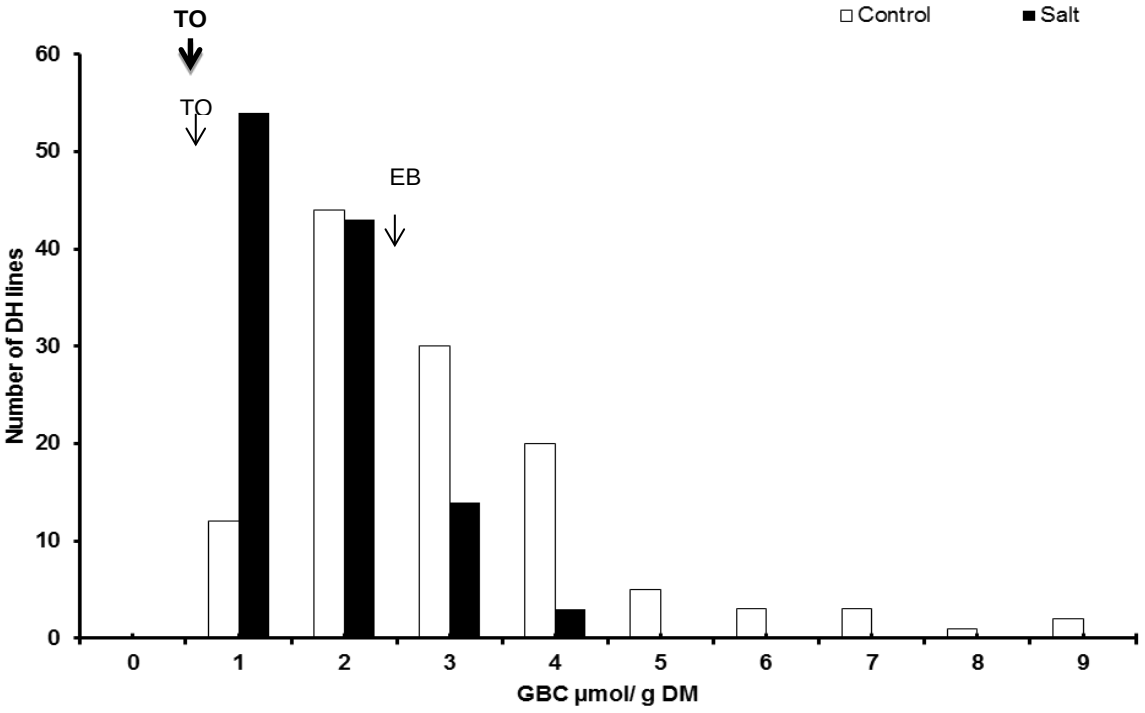
Appendix 13 continued from page 189



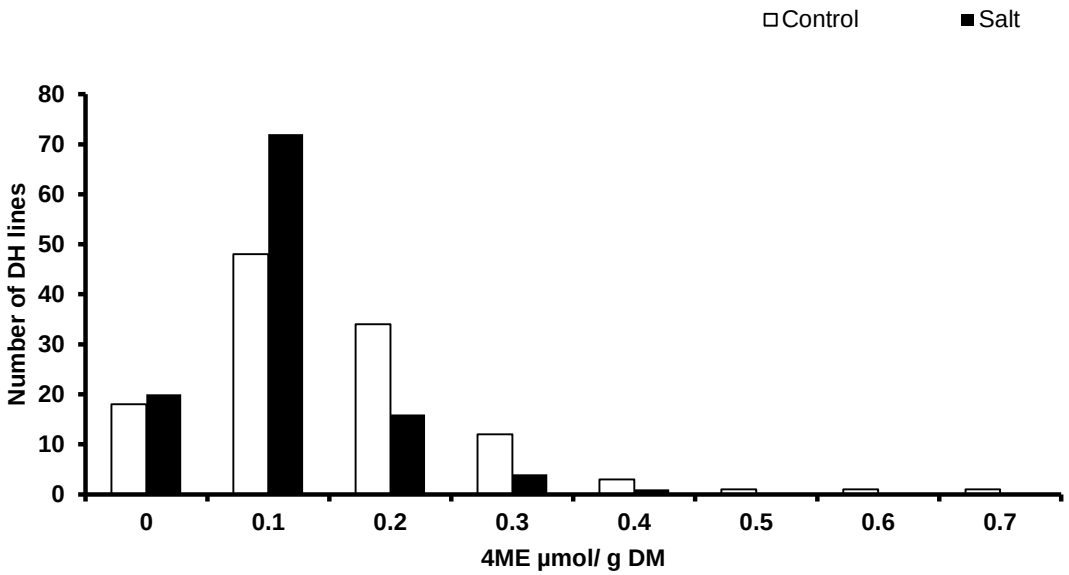


Appendix 13 continued from page 190

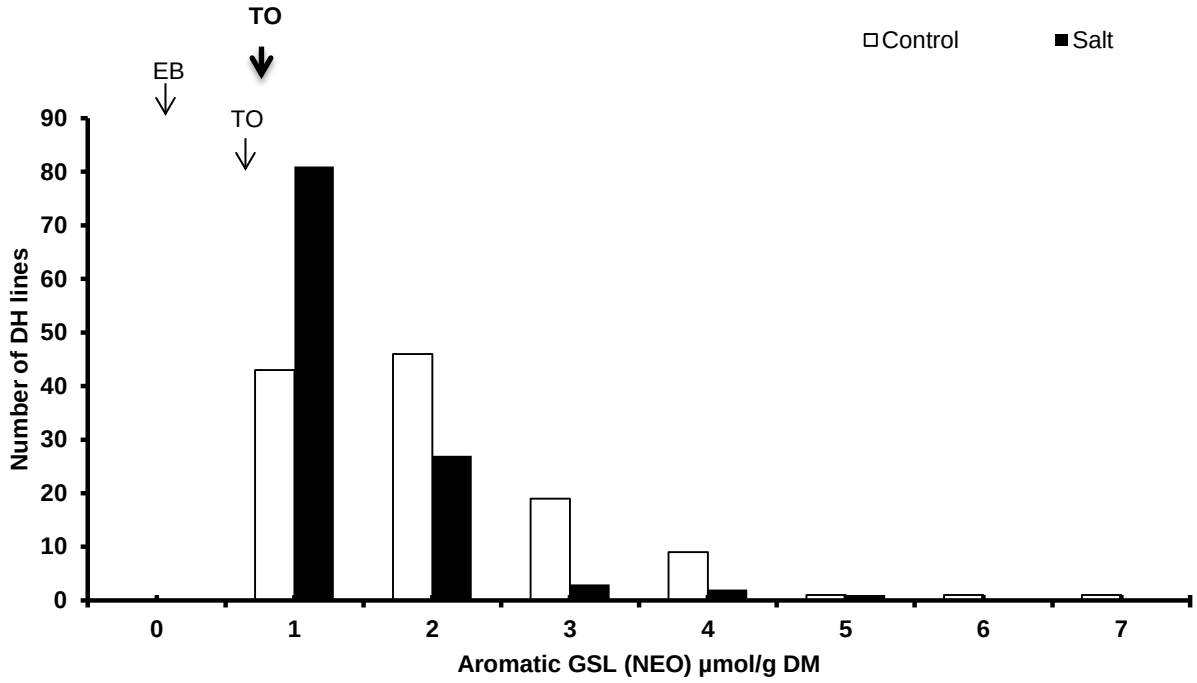




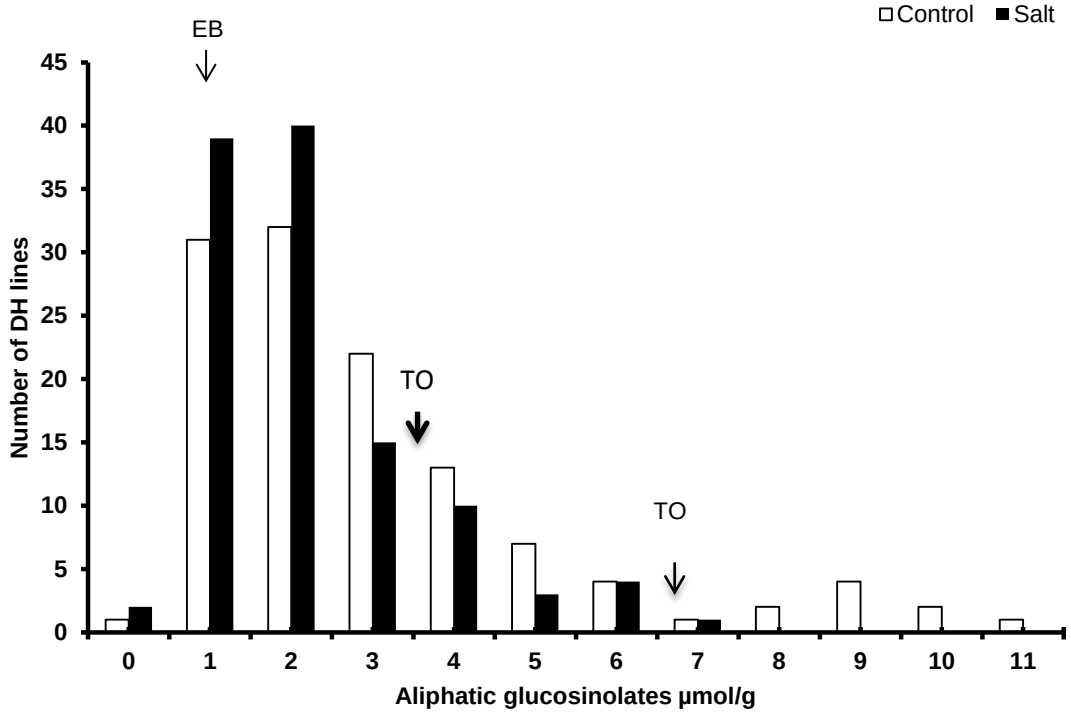
Appendix 13 continued from page 191

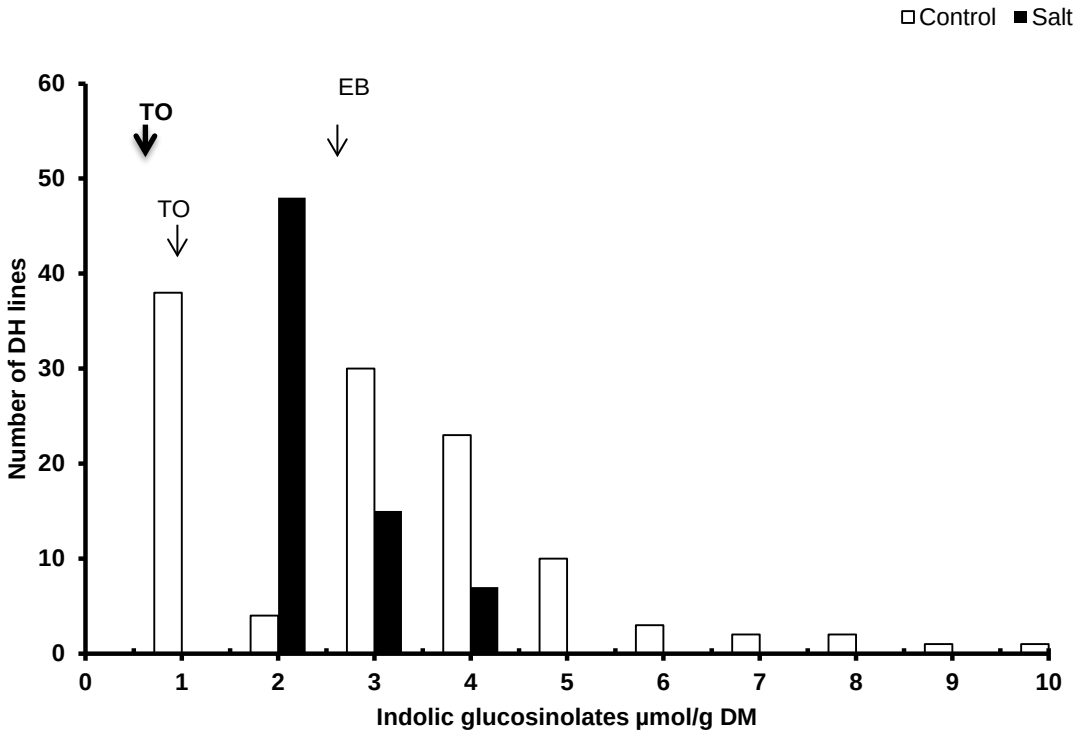


4ME was not detected in both parents



Appendix 13 continued from page 192





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Curriculum vitae of Yasser Shaaban Sayed Moursi

Biographical

Name	:	Yasser Shaaban Sayed Moursi
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Date of Birth	:	14/01/1981, Fayoum, Egypt
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Education

1998-2002	:	B.Sc. in Botany, Botany department, Faculty of Sciences, University of Fayoum Grade: Very Good
2006-2008	:	M.Sc. in Botany (Genetics and cytology) Botany department, Faculty of Sciences, University of Fayoum Grade: Excellent **Thesis title** Genetic diversity of *Brassica rapa* the progenitor of oilseed rape *Brassica napus*
2011-2014	:	Preparing for Ph.D. studies in Division of Plant Breeding, Georg-August- Universität Göttingen, Germany. **Thesis title** Genetic mapping of QTL controlling salt tolerance and glucosinolates in *Brassica napus* and *Brassica oleracea*

Academic records

2003-2008	:	Demonstrator (Teaching and Research Assistant), Botany department, Faculty of Sciences, University of Fayoum
2008-2014	:	Assistant Lecturer (Teaching and Research Assistant), Botany department, Faculty of Sciences, University of Fayoum

Awards

2010-2014	:	DAAD PhD Scholarships, Division of Plant Breeding, Faculty of Agricultural Sciences, Georg-August- Universität Göttingen, Germany

Attended workshops and training courses:

12-16.11.2012	:	Molecular biology techniques
13-17.02.2012	:	Training course in Statistics and STATISTICA, University of Göttingen, Germany
02-06.09.2013	:	Workshop on Molecular Techniques in Phytopathology, University of Göttingen, Germany

Attending conferences:

05-09.06.2012		The IRC 13th International Rapeseed Congress 201, Prague, Czech Republic
28.02-01.03.12	:	Breeding crops for sustainable agricultural production international conference. Justus -Liebig-Universität Gießen, Germany
18-20.09.2012	:	Genome Research Working Group Conference of the GPZ. Martin Luther University Halle-Wittenberg, Germany
19-21.09.2012	:	Resilience of agricultural systems against crises Conference. Georg-August- Universität Göttingen, Germany
24-26.09.2013	:	Georg-August- Universität Göttingen, Germany
11-13.02.2014	:	Conference of the Genome Research Working Group, Max Plant Institute for Plant Breeding Research. Köln, Germany.

Experiences:

- Methodology : Plant breeding methods, DNA isolation, gel documentation, genetic engineering, cytogenetic techniques, PCR, electrophoresis, molecular marker (RAPD, AFLP, SNPs), association analysis, QTL mapping.
- Software : Plabstat, Plabplan, STATISTICA, TASSEL 3.0, QTL network, Mapmaker 3.0, Mapchart 2.0, STRTUCTURE 2.3.4 for population genetic structure

Field of study:

- Quantitative genetics, Population genetics, Molecular plant breeding and genetics, and Cytogenetics.

Languages:

- Arabic (mother language)
- English
- German