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Analysis of complex inherited traits in maize (*Zea mays* L.) by expression profiling using microarrays

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Comparative Expression Profiling in Meristems of Inbred - Hybrid Triplets of Maize Based on Morphological Investigations of Heterosis for Plant Height

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LIST OF SPECIAL ABBREVIATIONS

BLAST	Basic Local Alignment Search Tool
Ct value	Concentration of DNA molecules multiplied by time
Cy3	Cyanine 3
Cy5	Cyanine 5
d / a ratio	dominance / additivity ratio
DEPC	Diethyl Pyrocarbonate
dNTP	deoxyribonucleotriphosphate
DMSO	Dimethyl Sulfoxide
eIF3e	eucaryotic translation initiation factor 3
EST	Expressed Sequence Tag
F_1	First filial generation, produced by crossing two parental lines
FDR	False Discovery Rate
gDNA	genomic Deoxyribonucleic Acid
GO	Gene Ontology
HPH	High-Parent Heterosis
INT	Internode lenght
Lowess	Locally weighted scatterplot smoothing
Mac1	Maize actin 1 gene
MDMV	Maize Dwarf Mosaic Virus
MPH	Mid-Parent Heterosis
NOI	Number of Internodes
qRT-PCR	quantitative Real Time Polymerase Chain Reaction
QTL	Quantitative Trait Locus
PCR	Polymerase Chain Reaction
PHT	Plant Height
RGA	Resistance Gene Analoque
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SCMV	Sugarcane Mosaic Virus
SOTA	Self Organizing Tree Algorithm
SSC	Standard Sodium Citrate
TIFF	Tagged Image File Format
TRIP	triplet

1 INTRODUCTION

During the past decade molecular biology and biotechnologies revolutionised the improvement of living organisms. A lot has been done in the research of crop plants utilising both, simplest conventional breeding methods and high-throughput molecular technologies, like for instance 454 sequencing or gene expression profiling by microarrays.

Nowadays available complete sequences of model organisms, such as *Arabidopsis*, rice, or almost fully sequenced maize, due to the preserved order of genes between species, enable the detection of homologous genes in more or less related organisms. Identification of gene functions becomes feasible. Moreover, promising steps are being done to understand molecular mechanisms controlling biological processes involved in the creation of complex traits in plants.

1.1 Maize - General information

Maize (*Zea mays* L.) is classified to the Kingdom Plantae, Subkingdom Tracheobionta, Superdivision Spermatophyta, Division Magnoliophyta, Class Liliopsida, Subclass Commelinidae, Order Cyperales, Family Poaceae (grass family), Genus Zea (corn), Species *Zea mays* (corn), Subspecies *Zea mays ssp, mays* (http://www.gramene.org/). The origin of maize is hypothesised to be derived from teosinte (*Z. mexicana* or *Zea mays* subsp. *parviglumis*), an ancient wild grass growing in Mexico and Guatemala. However, its origin from Asia or Andean highlands is still being discussed (Beadle, 1939; Galinat, 1988). Teosinte and *Tripsacum* are important genetic resources for desirable traits transfers (for example disease resistances) to cultivated maize (Mangelsdorf, 1961; Sehgal, 1963; Paliwal, 2000 a). Teosinte and maize cross freely, whereas *Tripsacum* does not hybridise with teosinte or maize under natural conditions. However, synthetic hybrids were produced between *Tripsacum* and maize and grown until maturity (Paliwal, 2000 b).

First indications on domestication of maize originate from ~7.500-12.000 years ago. The question, how maize was transformed from a weedy grass into a highly productive plant within such a short period of time is still unanswered (Harlan and Chapman, 1992). Domestication resulted in the improvement of maize's agronomical properties, such as increased vigour, yield and uniformity, however, caused loss of its ability to survive in the wild without human intervention in planting and harvesting. The involvement of early farmers

in maize evolution is likely (Longley, 1941 a, b; Kisselbach 1949; Beadle, 1939, 1978, 1980; Sprague and Dudley, 1988).

Maize is a monocotyledonous, cross-pollinating crop. Its genome is estimated to be ~ 2300-2700 Mb in size, distributed over 10 chromosomes 1500 cM in length (http://www.gramene.org/zea/maize_facts.html). Theories on the genome organization of maize range from a true diploid (Weber, 1986), to an amphidiploid and tetraploid species (Bennett, 1983; Wendel et al., 1986; Moore et al., 1995). Genome size and organization varies largely among maize subspecies. More than 80 % of the genome consists of repetitive DNA including retrotransposons. The absence of colinearity observed in some regions between inbred lines, mainly due to presence or absence of genes, as well as various positions of repetitive sequences was speculated to be a possible cause of heterosis (Bruner et al., 2005).

Due to C4 photosynthesis, maize is one of the most productive crop species, and is the best source of metabolizable energy in livestock feeding programs (Bajaj, 1994; Paliwal, 2000 a). Maize produces substantial amounts of carbohydrates, stored in the endosperm, high levels of glutamin acid and leucine-rich proteins but is a poor source of the essential amino acids lysine and tryptophan. The majority of maize proteins (> 50 %) in the grain fraction consists of zeins, which have very low amounts of these two amino acids.

Maize offers a large range of varieties, such as flint, dent, floury, waxy, pop, sweet and pod corn (Figure 1). Most important types for grain, fodder and silage are flint and dent. Flint type is characterised by round, hard and smooth kernels, endosperm made of hard starch, good germination rate, early maturing and fast drying after reaching physiological maturity. In contrary, dent kernels are bigger in size and more flat. Their endosperm is composed of soft starch, which requires extended drying time. Dents are higher yielding than flints but more susceptible to grain moulds and insects in the field and in storage (Paliwal, 2000 c). Flint-dent hybrids are of particular interest in breeding programs, as they combine favourable properties of diverse maize types in one cross. As a result, higher yielding hybrids with hard, rounded shape kernels, maturing early and drying slow, which is beneficial for short-season growth areas, are produced.

The multiple usage forms of maize, for food, feed, pharmacy or industry makes this crop particularly valuable for mankind (Eckerd, 2003).

Figure 1. The diversity of corn

Growth and reproduction of maize is simple, so that maize has been employed as model system for addressing several genetic questions. Its well-known genetics, structure and bilateral symmetry of vegetative parts divided into phytomers makes it particularly suitable for studies on phylogenetics, organogenesis, cell differentiation, and morphogenesis (Galinat, 1994; Maiti and Wesche-Ebeling, 1998). In summary, the usefulness of corn as crop and for genetic studies is mostly due to the open-pollination system, its hybrid vigour, multiple end uses and the broad range of environments for maize production.

1.2 Maize hybrid breeding

The interest in hybrid breeding programs on a large scale emerged from early reports of Shull (1908, 1909) and East (1908), when hybrid vigour and high uniformity of hybrids, developed from inbred line crosses were reported. Maize was the first major crop species where hybrid vigour was investigated, mainly due to physical separation of male and female flowers allowing for controlled crosses at large scale. The upcoming years brought establishment of double-cross hybrid production and introduction of the heterosis concept (Paliwal, 2000 e). The hybrid breeding programs initiated in Iowa and Illinois experimental stations expanded rapidly, from 10 % in 1935 to over 90 % till 1939, and were followed across entire United States and Canada (Lamb, 2000). The trend reached Europe in the early 1950s and started gaining further attention in developing countries of tropical and subtropical areas in 1960s. At the same time double-cross hybrids started to be replaced by higher yielding and even more uniform single-cross hybrids and further improvement of maize hybrids followed until the present, with more sophisticated methods and technologies being applied (Hallauer, 1999). Due to its adaptability and productivity, maize cultivation spread rapidly and is meanwhile the second most cultivated field crop worldwide after wheat based

on acreage with the tendency to receive the leadership in the near future (http://faostat.fao.org/default.aspx). The areas of maize cultivation are between 58° N in Canada to 40° S in Chile and Argentina, which demonstrates the high acclimatisation potential of maize to various growth conditions (Dowswell, 1996).

Major producers of corn are United States (280 million metric tons), China (131), Brazil (35), Mexico (21) and Argentina (21) (2005, FAO) (Meng and Ekboir, 2001). United States and China produce together ~ 60 % of the crop. The majority of the US corn is utilized as a food for livestock, forage, silage and grain (Paliwal, 2000 d; http://www.gramene.org/zea/maize_intro.html). The demand for maize as human food as well as maize production for livestock feeding is increasing worldwide. The global production of maize is predicted to increase by 50 %, with up to 79 % in developing countries and up to 93 % in Asia and Saharan Africa until 2020 (http://www.cimmyt.org/Research/-Economics/map/facts_trends/maizeft9900/pdfs/maizeft9900.pdf).

Hybrid breeding is not limited to cross-pollinated crops. It is rapidly spreading in self-pollinated crops, like cotton, rice, tomato and wheat (Paliwal, 2000 e). However, after over one century of investigations on this phenomenon the molecular background of hybrid vigour remains still to be discovered.

1.3 Heterosis - Definitions and Hypotheses

Shull in 1914, defined heterosis as the increase of size, yield and vigour, nowadays extended to the definition of the superiority of the F_1 hybrids over their parental inbreds manifested in quantitative characters and expressed as increased biomass, growth rate, fertility, resistance to diseases and insects as well as tolerance to abiotic factors (Keeble and Pellew, 1910; Bruce, 1910; Birchler et al., 2003; Meyer et al., 2004; Uzarowska et al., 2007). Hybrids are characterized by increased speed in cell division during growth. Anatomically the more rapid increase in size of hybrids was shown to be a result of greater cell numbers rather than cell size (Kiesselbach, 1922). As a result, negative heterosis is often found for flowering time in maize (Becker, 1993).

From the genetic point of view, hybrid vigour occurs when alleles from both parent inbreds are different and complementary. In order to predict the extent of heterosis in the hybrid the genetic distance between the parental inbred lines can be estimated. However, the relationship of increased genetic distance with increase in hybrid vigour holds only true to some optimal extent (Moll et al., 1965).

As early as in the year 1907 G.H. Shull underlined the positive correlation of hybrid vigour with the degree of dissimilarity in hybrid gametes. Richey, (1922), in his studies on heterotic groups in maize claimed higher performance of crosses derived from genetically or geographically distant parents, containing various endosperms. The phenomenon was called the superiority of inter-group over intra-group crosses and extensive experiments proving this thesis were carried out on maize (Kauffmann et al., 1982; Dudley et al., 1991; Dhillon et al., 1993), rice (Xiao et al., 1996) and rye (Hepting, 1978).

Three levels of hybrid vigour were proposed by Konarev in (1976): 1) heterosis at the molecular level, that considers the importance of increased DNA reduplication, transcription, translation, DNA extra copies formation, and the increased efficiency of enzymes and hormones in a heterotic organism, 2) heterosis at the metabolic and functional level, that enhances the effectiveness of regulatory systems through mitochondrial and plastid complementations. Mitochondrial complementation allows for increased coupling of oxidation to phosphorylation and escalates ATP synthesis (Sarkissian and Srivastava, 1969), 3) heterosis at the cell and organism level, which describes the increase in numbers of mitoses, increased growth of vegetative organs, synthesis and accumulation of nutrients, transport of metabolites for the yield formation and the interaction an complementation of cells, tissues and organs.

Heterosis is measured either as mid-parent heterosis (MPH), the difference between the hybrid and the mean of the two parents, or as high-parent heterosis (HPH), which refers to the difference between the hybrid and the parent with the highest trait value (Lamkey and Edwards, 1998).

Three hypotheses are proposed to explain heterosis: 1) **dominance hypothesis**, which is the masking of harmful recessive by superior dominant alleles in the heterozygous hybrid (Davenport, 1908; Bruce, 1910; Jones, 1917); 2) **overdominance hypothesis**, the superior phenotypic performance of the heterozygote as compared to both homozygous genotypes (Hull, 1945; Crow, 1948); 3) **epistasis hypothesis**, which is the interaction of favourable alleles at different loci contributed by the two homozygote parents (Williams, 1959; Li et al., 2001; Meyer et al., 2004).

1.3.1 Heterotic traits

1.3.1.1 PHT in maize as a model to study heterosis

As mentioned above heterosis is a complex inherited phenomenon influenced by many factors and the understanding of its molecular background requires extensive and focused investigations.

Plant height (PHT) is an excellent model character to study heterosis, as heterosis for PHT is substantial and can exceed 70 % in maize, giving the greatest effects after grain yield (Becker, 1993) (Figure 2). PHT is easy to determine, highly heritable (Lübberstedt et al., 1997; Lübberstedt et al., 1998), and closely correlated with important agronomical traits, like biomass production and forage yield (Lübberstedt et al., 1998; Niklas and Enquist, 2000). Moreover, significant correlations with PHT were found for number of leaves, grain yield, and flowering time (Troyer and Larkins, 1985). PHT is determined by complex interaction of many genes (Zsubori et al., 2002). Early morphological investigations suggested that 90 % of heterosis for PHT is due to an increased cell number, while 10 % is due to an increase in cell size (Kiesselbach, 1949).

Genes influencing PHT have been isolated (Jacobs, 1997; Yamauchi et al., 2004), including rice *d1*, the constitutive GA response gene *spy* in *Arabidopsis*, as well as the GA-deficient mutant genes in maize *d1-3, d5*, *D8/9* and *An1* (Ogawa et al., 1999; Olszewski et al., 2002). The simple dominant dwarfing mutation *D8/9* results in plants with significantly reduced internode length but unchanged internode number, not responding to exogenous GA application (Harberd and Freeling, 1989; Milach et al., 2002). The *Dwarf* 1 (*d1*) mutation in rice is characterized by a defect in the signalling pathway mediated by a G protein (Ashikari et al., 1999). However, it is unknown whether these genes are involved in the phenomenon heterosis for PHT.

Figure 2. Hybrid vigour in maize. Two inbred lines B and H besides their hybrid (BxH) in the middle (Dr. Christina Ingvardsen)

1.3.1.2 Oligogenic resistance to SCMV - example of epistatis

About 80 % of resistances to specific plant pathogens are inherited by single dominant or partially dominant genes (Kang et al., 2005). However, overdominant inheritance of resistance has also been reported (Delaney et al., 1998). Moreover, cases when resistance segregates as recessive trait or is controlled by epistatic interaction of two non-allelic genes are known (Roelfs, 1988; Crute and Pink, 1996). Additive-by-additive epistasis for resistance to corn leaf rust was suggested as major source of variation in sweet corn (Randle et al., 1984). Similar effects were reported between two loci for resistance to spindle streak mosaic virus in wheat (Van Koevering et al., 1987). Epistasis between two QTLs was identified for resistance to rice yellow mottle virus and cucumber mosaic virus in pepper (Caranta et al., 1997; Ahmadi et al., 2001). Recently, substantial epistasis between two introgressed genome regions for SCMV resistance has been found (Xing et al., 2006). Due to this fact, it is assumed that oligogenic SCMV resistance can be considered as a model to study heterosis.

Viruses are among the most infective plant pathogens (Smith and White, 1988). The largest group includes potyviruses (Family *Potyviridae,* Genus *Potyvirus)*. In maize, the most important virus parthogens are the worldwide spread sugarcane mosaic virus (SCMV) and the taxonomically related potyviruses maize dwarf mosaic virus (MDMV), Johnsongrass mosaic virus (JGMV), and sorghum mosaic virus (SrMV) (Barnett, 1991; McKern et al., 1991; Shukla et al., 1992 a, b; Zhong et al., 2005). SCMV is a positive-sense ssRNA potyvirus that

causes significant yield losses in susceptible genotypes of maize, sugarcane and sorgum (Fuchs and Günzig, 1995; Xia et al., 1999). MDMV, except of infecting all corn types, has the ability to infect other grass species, such as annual, winter annual and perennial. Similar symptoms, host range, physical / physicochemical and transmission properties of SCMV and MDMV viruses were observed (Jiang and Zhou, 2002). SCMV is notably harmful in Europe and China, while MDMV mostly in southern US Corn Belt (Louie et al., 1991). MDMV affects both the growth and the yield of maize.

Disease symptoms are mosaic symptoms on leaves, chlorosis, leaf reddening, necrosis, stunting and plant weight reduction, differing in mosaic expression between strains (Tu and Ford, 1969 a, b; Fuchs and Günzig, 1995; Comstock and Lentini, 2002) (Figure 3).

Figure 3. Disease symptoms for SCMV (on the left) and MDMV (on the right) infection

Potyviruses are transmitted in a non-persistent manner by aphids (*Aphididae*: *Dactynotus ambriosiae, Melanaphis sacchari, Rhopalosiphum maidis,* among others) and the host range is mainly restricted to the members of the *Poaceae* family (Jain et al., 1998; Charpentier et al., 1956; Quint et al., 2002). Due to this fact, chemical control appears insufficient and the control of diseases most promising by using resistant varieties.

SCMV resistance is typical example of epistatic gene interaction, as two major SCMV resistance genes, *Scmv1* (two linked genes *Scmv1a/b* mapped on chromosome 6S) and *Scmv2* (chromosome 3L), are essential for full resistance (Lübberstedt et al., 2006; Xing et al., 2006) (Figure 4). *Scmv1* suppresses symptoms at all developmental stages, whereas *Scmv2* is involved at later stages of infection (Xia et al., 1999; Dußle et al., 2000). Three additional minor QTLs were identified on chromosomes 1, 5 and 10 (Xia et al., 1999).

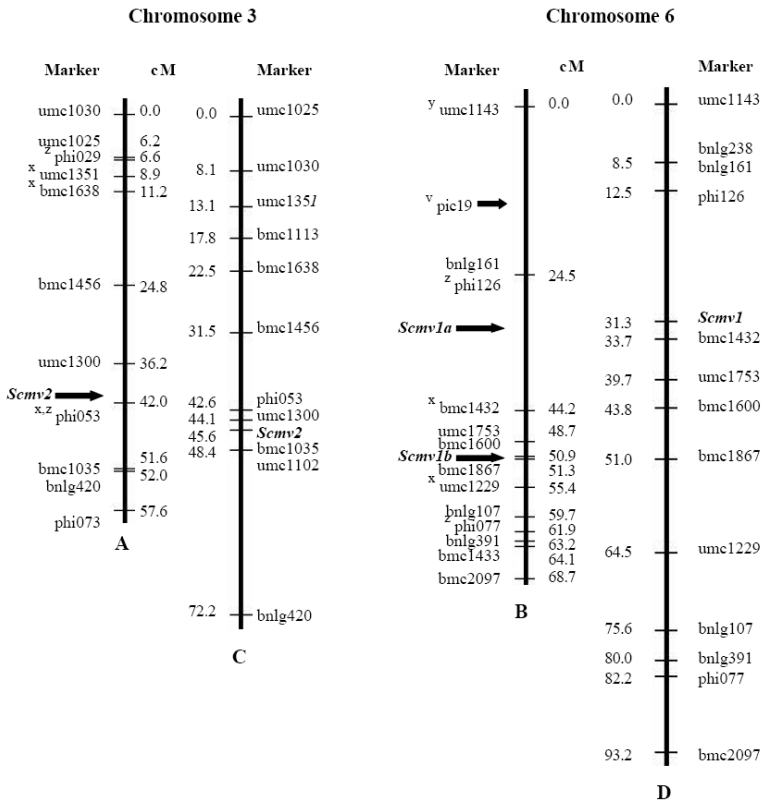

Figure 4. The genetic map of chromosome 3 and 6 in maize, obtained by linkage analysis of F_3 lines of a cross F7 and FAP1360A with SRR markers (Dussle, 2002)

One major *Mdmv1* gene, providing resistance to MDMV strains A, B, D, E and F was mapped to the same region of chromosome 6S as *Scmv1* using RFLP markers (McMullen et al., 1989; Melchinger et al., 1998; Louie et al., 1991). Other reports claim one to five MDMV resistance genes causing resistance reaction (Roane et al., 1977; Findley et al., 1984; Rosenkranz et al., 1984; Mikel et al., 1984; Louie et al., 1991). Moreover, resistance genes against wheat streak mosaic virus, *Wsm1* and *Wsm2* were detected in the *Scmv1* and *Scmv2* genome regions, respectively (Redinbaugh et al., 2004).

1.4 Molecular breeding for improvement of quantitative traits

Most of the achievements in plant productivity and quality increase of crops have been obtained by practical breeders. However, classical breeding methods are no longer sufficient to keep pace with the growing demand for higher yielding plants, crops free of diseases, crops resistant to environmental stresses or containing valuable compounds. The choice of proper technologies for improvement of complex inherited traits is a key point in successful breeding programs.

1.4.1 DNA markers

DNA marker technology in plants was first established in the 1980s, nowadays delivering thousands of markers for major crop species available in public databases. DNA markers provide the connection of plant biology with plant breeding, being an efficient indirect selection tool for tagging of agriculturally important genes, genetic assessment in plants and the dissection of complex traits with the quantitative mode of inheritance (Paterson et al., 1988; Stuber et al., 1992).

The usage of restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and random amplification of polymorphic DNA (RAPD) markers for the prediction of hybrid performance was successful in finding the agreement of genetic distances with the pedigree data for assigning inbred lines into heterotic groups. However, markers have so far not been effective in predicting hybrid vigour (Melchinger et al., 1990; Smith et al., 1990; Stuber, 1994 a; Dubreuil et al., 1996; Lanza et al., 1997; Ajmone-Marsan et al., 1998; Melchinger, 1999; Benchimol et al., 2000). The increase in efficiency for prediction of hybrid performance on the base of genetic diversity of parental genotypes was also reported by Melchinger, (1999). The connection was, however, loose for lines from different heterotic groups. Comprehensive studies carried out on maize and other crop species showed the genetic distance between parental lines to be positively correlated with hybrid performance, but not necessarily being an indicator for the extent of heterosis (Lee et al., 1989; Smith et al., 1990; Lanza et al., 1997; Ajmone-Marsan et al., 1998; Benchimol et al., 2000).

1.4.2 Quantitative Trait Loci

Molecular markers can be utilised in the analysis of complex inherited traits in crops for quantitative trait loci (QTL) mapping. The objective of QTL mapping is to identify marker closely linked to genes underlying quantitative traits and having significant individual effects on the phenotype (Lamb, 2000). The knowledge about the number of QTLs explaining variation in the phenotypic trait provides the information about the genetic architecture of a trait. For complex traits, molecular markers closely linked to QTLs must be identified to map the regions were the gene specifying a quantitative trait is located (Helentjaris and Heun, 1994). Numerous QTL studies on quantitative traits in maize have been performed, such as on resistance to *Ustilago maydis*, drought tolerance, morphological differences between maize

and teosinte, search for associations with heterosis and genotype x environment interactions (Edwards et al., 1987; Abler et al., 1991; Dudley, 1993; Knapp, 1994; Beavis and Keim, 1995; Lee, 1995; Dubreuil et al., 1996; Sari-Gorla et al., 1999; Lübberstedt et al., 1998). Tsaftaris, (1995) suggested that QTLs important for heterosis likely code for regulatory proteins, and that these proteins may control structural genes involved in heterosis manifestation.

1.4.3 Marker-Assisted Selection

Marker-assisted selection (MAS) is undoubtedly one of the most beneficial applications of molecular markers closely linked to QTLs. MAS allows indirect but highly accurate early selection for a trait or combination of traits (Stuber et al., 1992). Since the majority of relevant agronomic characters are inherited quantitatively the genetic improvement of such traits is time and cost-consuming. The possibility of MAS application at any step of plant development, and the selection independent from the environment seems advantageous as compared to classical phenotypic selection (Knapp, 1998; Yousef and Juvik, 2001). MAS is most successful for simply inherited traits, however, examples of its success for complex traits have been reported (Francia et al., 2005). Applications of MAS in various plant species are given in Table 1.

Table 1. The utilisation of marker-assisted selection in plant breeding

Reference	Plant	Application
Stuber, (1994 b)	Maize	Transfer of QTLs for hybrid grain yield to elite maize inbred lines
Ribaut and Bertrán, (1999)	Maize	Improvement of polygenic traits by single large-scale MAS
Ribaut et al., (2002)	Maize	Enhancement of drought tolerance in maize
Davierwala et al., (2001), Toenniessen et al., (2003)	Rice	Resistance screening to bacterial blight
Howes et al., (1998)	Wheat	Improvement of wheat breeding programs
Han et al., (1997)	Barley	Malting quality study
Arru et al., (2003)	Barley	Improvement of resistance against leaf stripe in barley
Schneider et. al., 1997	Bean	Optimisation of drought resistance
Hämäläinen et al., (1997)	Potato	Investigations of extreme resistance to potato virus Y
Lascape et al., (2003)	Cotton	Improvement of fiber quality
Barone, (2003)	Tomato	Resistance to pathogens
Cregan et al., (2000)	Soybean	Improvement of resistance against soybean cyst nematode

1.5 Application of functional genomics in dissection of complex inherited traits

The knowledge of the function of genes benefits plant breeding. It can be achieved by functional genomics, which associates genes identified by genome analysis with phenotypic traits (Hieter and Boguski, 1997).

Modern technologies including sequencing, transposon tagging, developmental genetics, transformations, gene cloning, expression profiling or RNA interference are being improved in order to generate better performing living organisms (Peterson and Bianchi, 1999). The rapid increase in application of microarray technology in recent years tells all about its usefulness and advantageous character in modern biotechnology research (Li et al., 2002; Marshall et al., 2004).

1.5.1 Expressed Sequence Tags

Gene discovery in most plants is nowadays based on expressed sequence tags (ETSs). ESTs are single-pass sequenced cDNAs from an mRNA population (Kohane, 2003). They are derived from defined cells or tissues and can be mapped to specific chromosome locations as genetic marker. Because of cDNA complementarity to mRNA, the ESTs represent portions of expressed genes. Moreover, they allow quick identification of putative gene function by similarity to already known entries. The frequency of EST recovery for individual genes is being used for the estimation of expression patterns of individual genes (Fernandes et al., 2002). Large-scale EST sequencing has greatly contributed to gene discovery. A total amount of 43.170.777 EST entries for 1.313 various organisms have been developed and deposited in public databases (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html). Within them 1.276.207 ESTs are available for *Arabidopsis,* 1.211.447 for rice, 1.161.241 for maize, and 1.050.131 ESTs for wheat. ESTs are the fundament of the majority of microarray-based technologies utilising cDNA spotted probes. However, the disadvantage of arrays spotted with ESTs is the representation of only a portion of isolated genes, in contrary to oligo-arrays, where every gene from the fully sequenced genome can be predicted (Chen et al., 2004).

1.5.2 Expression profiling

The sequence of a gene is not necessarily informative for the gene function. Around 30 % of *Arabidopsis* genes possess no homology to sequences of known or hypothetized function (Wisman and Ohlrogge, 2000). Due to the fact that gene expression correlates with function, it is possible to follow the up- and down-regulation of genes in a given physiological state by using high-throughput technology developed in the mid-nineties, microarrays. Their unique ability to measure the expression of tens of thousands of genes in parallel, thus generating global gene expression patterns for different developmental stages, tissue types, or environmental factors is of particular importance in supplying new insights into already described, as well as uncharacterized genes (Jain, 2000; Slonim, 2002; Schnable et al., 2004). The miniaturisation, integration and parallel-processing are the major advantages changing the scale of quantification as compared to older and less sophisticated techniques, resulting in acquisition of huge amounts of data in a relatively short experimental time (Schena, 2003).

Basic microarray technologies comprise oligonucleotide arrays (short 25-25bp or long >50bp oligos) utilised mainly for expression profiling studies of known sequences, cDNA arrays (500-2500 pb long sequences) for organisms with not fully sequenced genomes, protein arrays and tissue arrays. cDNA arrays account for ~65 % of the microarray usage, oligo arrays for ~26 %, and tissue and protein arrays for ~8 %. The majority of application utilising array platform covers gene expression profiling (~81.5 %) and genotyping (~12 %), whereas only few percent of arrays are utilised for tissue and protein assays (Müller and Röder, 2006).

Most advanced microarray research in plants is being developed for the dicotyledonous model organism *Arabidopsis*. Oligonucleotide Affymetrix arrays have been used for instance to determine expression profiles in various organs, to identify common signalling pathways and conserved genes or to compare specific tissues between *Arabidopsis* and other species (Desprez et al., 1998; Ruan et al., 1998; Horvath et al., 2003; Lee E. J. et al., 2004). Moreover, microarrays developed for *Arabidopsis* can additionally be utilised in studies on *Brassica*, due to high, up to 90 % homology of sequences between those two species (Lee H. S. et al., 2004). *Arabidopsis* arrays containing whole genome probes or fragments of chromosomes have been also produced (Chen et al., 2004; Redman et al., 2004).

For monocotyledonous maize, microarrays have been utilised to estimate dominance between inbred lines and their hybrids (Keller et al., 2005), characterise the expression of genes in relation to SCMV resistance (Shi C. et al., 2005), determine gene expression levels in developing embryos (Lee et al., 2002), analyse gene responses to UV radiation (Casati and

Walbot, 2003) or to determine the validity of comparisons among mutants in different maize lines (Ma et al., 2006). Maize unigene microarrays are produced by Schnable Lab and contain 15 680 spots, out of which 14 117 being unique (http://www.plantgenomics.iastate.edu/-maizechip/).

The array technology has recently been applied in rice (NSF Rice Oligonucleotide Array Project), to fabricate arrays containing 45 000 *Oryza* oligonucleotide sequences (http://www.ag.arizona.edu/microarray/, http://www.ricearray.org/), in *Medicago*, to develop arrays containing 16 000 oligonucleotide sequences or in potato, where over 15 000 cDNAs are spotted onto the glass slide (http://www.tigr.org/tdb/potato/microarray_desc.shtml).

In other plant species, microarray technology has been adopted for gene discovery, for instance to compare mutant to wild-type plants, to monitor organism-level responses to environmental stimuli, to characterize genes for strawberry flavour and to discover novel genes between near-isogenic lines by analysis of mutant and normal lines in soybean (Desprez et al., 1998; Aharoni et al., 2000; Reymond et al., 2000; Helliwell et al., 2001; Seki et al., 2001; Vodkin et al., 2004). Non-plant microarray applications include such areas as epidemiology (identification of pathogens and screening for resistances to infections), pharmacology (drug development and safety), oncology (cancer prognosis and diagnosis) or clinical research (detection of mutations and inherited disorders) (Jain, 2000).

The complete microarray system includes sample preparation, array generation, sample hybridization, data handling and interpretation. Sample preparation includes the selection of appropriate biological material, based on the aim of the experiment and its preparation and labelling with radioactive probes (P^{32} or P^{33}), biotin / dioxigenin (half-antigens) or with most commonly employed fluorescent labels (Cy2, Cy3, Cy5 or analoques). Array generation implies the decision of which, cDNA or oligo samples should be spotted on the array and its construction by one of the spotting methods, for instance photolitography, inkjet-controlled synthesis, mechanical spotting (touch spotting) or non-touch spotting (piezo-electric point). Hybridization and scanning generate images for further data handling and the application of an appropriate bioinformatic tool for data analysis and further interpretation is of significant importance to obtain trustworthy results (Bowtell, 1999; Schena, 1999; Allison et al., 2006; Müller and Röder, 2006).

1.6 Objectives of the work

Characters, such as plant height and SCMV resistance were chosen for this study to investigate complex inherited traits in maize.

The objectives of the work were to: 1) investigate plant height heterosis under various experimental conditions and within different combinations of inbred-hybrid triplets, 2) identify those stages and parts of maize plants explaining most of the PHT differences between inbred lines and their hybrids, 3) identify differentially expressed genes in relation to heterosis for plant height, 4) determine the consistency of expression patterns between inbred parent-hybrid triplets in view of differing degrees of relatedness of triplets, 5) identify differentially expressed sequences related to resistance to SCMV virus, 6) follow the expression patterns of virus infection over time points, and 7) to investigate the potential and reliability of the combination of two expression profiling technologies, such as microarrays and quantitative real time RT-PCR in the identification of truly differentially expressed genes for complex inherited traits.

2 MATERIALS AND METHODS

2.1 Morphology and expression profiling studies

2.1.1 Plant materials

For morphological investigations of heterosis, maize (*Zea mays*) inbred lines 002, 005 (European flints), 250 (Iowa Stiff Stalk dent), 301 (Lancaster dent), their inter-pool (002x301, 005x250, 250x002, 301x005) and intra-pool hybrids (002x005, 250x301) with reciprocals were obtained from Prof. Dr. A.E. Melchinger, University of Hohenheim, Germany. One reciprocal for each hybrid was chosen for further experiments based on the germination and growth rates.

The two parental components and their hybrid were compared for the hybrid vigour analyses and hence they are called triplet (TRIP) in this study (Table 2).

Table 2. Triplets description

Triplet	Parental inbreds & hybrid
TRIP1	002, 301 & 002x301
TRIP2	005, 250 & 005x250
TRIP3	250, 002 & 250x002
TRIP4	301, 005 & 301x005
TRIP5	002, 005 & 002x005
TRIP6	250, 301 & 250x301

Plants were grown in two separate greenhouse experiments at the Technical University of Munich, Freising, Germany in 2004 and 2005, until maturity (BBCH uniformal decimal growth stage scale 65 - 69 for maize: end of flowering, Lancashire et al., 1991). They were planted in a split plot design with the six hybrids and four inbreds in separate blocks, where hybrid vs. inbred was the main plot factor and the genotypes the sub plot factor with two replications. Ten genotypes with 5 plants per genotype were sown in each greenhouse experiment, with a density of 9 plants per m^2. Seeds were sown in multi-pots and the reporting of plants was conducted 2 weeks after sowing into 2 l pots, and 4 weeks after sowing into 10 l pots. Plants were fertilized once a week with 200-300 g / 100 l H_2O plant fertilizer Flory 3 Grün, (Euflor GmbH, Munich, Germany). The temperature in the

greenhouse was set to 24° C during the day and 18° C at night. Plant height (PHT), number of internodes (NOI), length of internodes (INT) and width of internodes were measured on five representative plants per plot, at the end of flowering, numbering internodes from top to bottom (internode 1 = tassel). Only above ground internodes were measured. Width of internodes was measured only in 2004 and was excluded from further analyses due to low heritability.

For expression profiling analyses, four inbred lines (002, 005, 250, and 301) and their four inter-pool hybrids (002x301, 005x250, 250x002, 301x005) were grown in the greenhouse at the Research Centre Flakkebjerg, Denmark in two seasons with two replications per season (winter 2003, summer 2004) at 24° C during the day and 18° C at night temperature for 21 to 23 days (BBCH uniformal decimal growth stage scale for maize 14 to 15: 4 to 5 leafs unfolded). Plants were randomized in a complete block design with 25 and 40 plants per genotype for inbreds and hybrids, respectively. Plants were watered automatically once in the morning and once or twice in the afternoon (according to the amount of sunlight) with a fertilizer from two sources containing, first: 3 kg of $Ca(NO_3)_2$, 15.5 % N in 40 l of water, second: N – P – K 14-3-23 (3 kg in 40 l of water) plus 400 g of microelements: B – 0.23 %, Cu – 0.14 %, Fe – 1.32 %, Mn – 0.50 %, Mo – 0.05 % and Zn – 0.18 %.

Upper stem meristems were harvested 21 - 23 days after sowing with a sterile scalpel and placed into 1.5 ml Eppendorf tubes, subsequently quick-frozen in liquid nitrogen and stored at -80° C. The localization of the upper stem meristem in a 3-week old plant was done using a stereo-microscope. Meristems from the same genotype within one replication were pooled to obtain sufficient mRNA quantities.

Field morphological experiments were carried out in Freising and Pulling, Germany, in 2004 and 2005. Plants were grown in a split plot design with the six hybrids and four inbreds in separate blocks, hybrid vs. inbred being the main plot factor and the genotypes the sub plot factor with three replications. Ten genotypes with 50 plants per subblock per genotype were sown. PHT, NOI, INT and width of internode measurements were conducted as in the greenhouse experiments, but on three representative plants per plot.

Four near-isogenic homozygous maize (*Zea mays* L.) genotypes: F7 $^{SS/SS}$ (highly susceptible), F7 $^{RR/RR}$ (fully resistant), F7 $^{SS/RR}$ (resistant at *Scmv1* on chromosome 6) and F7 $^{RR/SS}$ (resistant at *Scmv2* on chromosome 3) were produced at the Research Centre Flakkebjerg, Denmark and utilized in the virus resistance study. The resistant near-isogenic line F7 $^{RR/RR}$ (with FAP1360A introgression at the two target regions) was derived from the

cross between the flint line F7, susceptible to SCMV, and the Early-maturing European dent line FAP1360A, completely resistant to SCMV, seven times backcrossed to F7 and three times selfed. The modified F7 line was called 10940. The sublines F7 $^{RR/SS}$ and F7 $^{SS/RR}$ were produced from plants carrying resistance alleles (*Scmv1*, *Scmv2*) fixed at one *Scmv* QTL region, and with F7 alleles (*Scmv1*, S*cmv2*) fixed at the second region.

Plants were grown at the Research Centre Flakkebjerg, Denmark, under controlled greenhouse conditions for 14 days before virus inoculation, at 24° C during the day and 18° C at night in five blocks (= time points) with four biological replications each, with the four near-isogenic genotypes within replication and eight plants per genotype in a split plot design. An additional block with mock control plants was included.

After 14 days, leaf samples for the SCMV experiment were harvested at time point 1 (before infection), followed by virus inoculation and harvesting at time point two (one hour after infection), time point three (six hours after infection), time point four (twelve hours after infection) and time point five (twenty four hours after infection).

The SCMV inoculation mixture was prepared from four to five young leaves of the SCMV infected susceptible F7 genotype displaying typical mosaic symptoms, homogenized in five volumes of a 0.01 M phosphate buffer (pH 7.0) and well mixed with carborundum. The inoculation method was a mechanical rub inoculation. Mock control plants were inoculated with water and harvested before inoculation of the plants with the SCMV virus. For each of the eight plants per plot, four young leaves were infected and harvested into two pools containing four plants, subsequently quick-frozen in liquid nitrogen in aluminium bags and stored at -80° C.

Solutions utilized:

0.01 M phosphate buffer (pH 7.0)
0.2406 g KH_2PO_4 0.543 g Na_2HPO_4 500 ml H_2O

2.1.2 RNA isolation and sample preparation

mRNA was isolated using DynaBeads oligo$(dT)_{25}$ (Dynal Biotech, Oslo, Norway). 1.5 ml of lysis / binding buffer was applied into the 2 ml tube containing ~200 mg of raw grinded material, homogenized with a microtube pestle and centrifuged at full speed for 5 min at 4° C. A 2 ml Eppendorf tube with 250 µl of aliquoted DynaBeads was placed for 30 sec into magnetic particle concentrator (MPC) (all steps with supernatant removal were performed in MPC) to remove beads storage solution and treated with 500 µl of lysis / binding buffer. The buffer was subsequently discarded and DynaBeads were ready to use. The crude extract (supernatant) from the centrifuged sample was applied to the beads, avoiding floating particles. These samples were well-mixed with the pipette and left for 3-5 min for incubation at RT, with frequent inversion of the tube for beads suspension. The supernatant was discarded and DynaBeads were washed with washing buffer A. At this step the next crude extract from the same genotype was ready to be applied to the beads and the procedure was repeated 3-4 times, for the purpose of obtaining sufficient amounts of mRNA. Four to six tubes were handled in parallel following the protocol. After the last sample was applied to the beads they were washed twice with washing buffer B and stored at 4° C until performing reverse transcription reaction.

Reverse transcription was performed with Superscript II (Invitrogen GmbH, Karlsruhe, Germany). DynaBeads with mRNA polyA coupled to dTTT immobilized to beads were 3-times washed in 250 µl of ice-cold first strand buffer and transferred into 1.5 ml Eppendorf tubes between washings. 30 µl of RT-mix per sample was applied and samples were incubated 1 hour in a rotary oven at 42° C. Thereafter samples were washed twice with 300 µl TE buffer, resuspended in fresh 300 µl TE buffer and incubated 6 min at 95° C in order to remove the rests of the RNA annealed to first strand cDNA. For storage at 4° C samples were resuspended in 200 µl TE buffer.

Second strand synthesis was conducted by Klenow DNA polymerase I (Fermentas Life Sciences, St. Leon-Rot, Germany) on DynaBeads with incorporation of aa-dUTP's. TE buffer was discarded and the sample was resuspended in 23.5 µl of water and 4 µl of 50 µM nanomer random primer-mix (N9B). Incubation was carried out at 95° C for 3 min, followed by primer annealing at room-temperature for 5 min, briefly suspending the beads by rolling. Klenow reaction mix was applied and the probe was incubated 1.5 hours in a rotary oven at 37° C. After second strand synthesis the probe was washed twice with 300 µl of washing buffer BX and resuspended in 40 µl of water, followed by 5 min incubation at 95° C. After

denaturation the supernatant was quickly eluted to a new tube and stored at ice. Second strand synthesis was repeated 2-4 times for each sample and the second strand cDNAs from each genotype were pooled and measured on the spectrophotometer (Genesys 10 Bio, Thermo electron corporation, Madison, USA). The absorption was measured at 260 nm in a 50 µl quartz cuvette and the concentrations of second strand cDNAs were calculated as follows:

$$\text{pmol nucleotides} = \frac{(OD_{260} x vol(\mu l) x 37 ng/\mu l x 1000)}{324.5 pg/pmol} \text{ (vol = 80 µl).}$$

Second strand cDNA probes were labelled with Cy3 and Cy5 (Amersham Pharmacia, Piscataway, USA), depending on the hybridization design. The same amounts of Cy3 and Cy5 labelled samples were VacuFuge dried (45° C) and carefully resuspended in 10 µl of 0.1 M sodium bicarbonate (pH 9.0). The dried, -20° C stored Cy3 / Cy5 dyes were resuspended in 2 µl of DMSO in parallel. Samples were mixed and incubated at 28-30° C in a rotary oven in the darkness. The labelling reaction was stopped after 1 hour by adding 38 µl of 100mM NaOAc (pH 5.2) and unincorporated dyes were removed with QiaQuick PCR purification kit (QiaGen AG, Hilden, Germany) according to manufacturer's recommendations. The amount of labeled product was measured spectrophotometrically in a 50 µl quartz cuvette for the wavelengths 260 nm (DNA), 550 nm (Cy3), and 650 nm (Cy5). The incorporation of Cy3 and Cy5 was calculated like following:

$$\text{Cy3 (pmol)} = \frac{OD_{550} x vol(\mu l)}{0.15}, \text{ and Cy5 (pmol)} = \frac{OD_{650} x vol(\mu l)}{0.25}$$

Solutions utilized:

Lysis / binding buffer 4° C	**Washing buffer A 4° C**	**Washing buffer B RT**	**TE buffer pH 7.5 RT**
100 mM Tris – HCl, pH 7.5	10 mM Tris – HCl, pH 7.5	10 mM Tris – HCl, pH 7.5	10 mM Tris-HCl, pH 7.5
500 Mm LiCl	0.15 Mm LiCl	0.15 Mm LiCl	1 mM EDTA, pH 8.0
10 mM EDTA	1 mM EDTA	1 mM EDTA	
1 % LiDS	0.1 % LiDS		
5 mM DTT			

RT-mix	**1 x (µl)**
water	17.5
5 x first strand buffer	6.0
DTT (0.1 M)	3.0
dNTP (each 25 mM)	1.5
RNaseOUT (40 U / µl)	1.0
Superscript II (200 U /µl)	1.0
Total	30.0

Klenow-mix	**1 x (µl)**
10 x Klenow buffer	4.0
dNTP-lowT mix – A,C,G: 20 mM, T: 6.5 mM	1.0
aa-dUTP, 2 mM	6.6
Klenow (10 U / µl)	1.0
Total	12.6

First strand buffer
55 mM Tris-HCl, pH 8.3 82.5 mM KCl 3.3 mM $MgCl_2$ Autoclave

Washing buffer BX
0.1 M phosphate buffer, pH 7.5: 4.33 ml 1 M K_2HPO_4 + 0.67 ml 1 M KH_2PO_4 and adjust to 50 ml with water. Mix 5 ml 0.1 M phosphate buffer and 2.5 ml 20 x SSC, adjust to 50 ml with water.

0.1 M Sodium bicarbonate, pH 9.0
For 100 ml: 0.84 mg $NaHCO_3$ dissolve in 90 ml of water and adjust to pH 9.0. Add water till 100 ml. Prepare fresh and store aliquits at -20° C for few months.

2.2 Array type

2.2.1 Unigene arrays

The high density cDNA unigene 12 k microarrays produced by the laboratory of Prof. Dr. P. Schnable (Iowa University, Ames, USA) were utilised for heterosis investigations. The array contained 11.827s maize ESTs clustering into 9.841 unigenes, which accounted for ~20 % of the about 50.000 maize genes (http://www.plantgenomics.iastate.edu/maizechip/). 11.027 genes were spotted once, 391 in duplicate and 6 in triplicate (Shi C. et al., 2005). The collection was derived from fifteen EST libraries from a variety of organs and tissues, like immature leaf, endosperm, ear tissue, root, tassel primordial, anther and pollen, 14 day immature embryo, mixed stages of embryo development, mixed adult tissues, seedlings and silks. EST libraries included also various treatments, like plants grown under normal environmental conditions, and "stressed shoot libraries". ESTs were spotted on Ultra GAPS Slides (Corning) coated with Gamma Amino Propyl Silane to obtain a high-quality DNA-binding surface. Slides were received from Dr. Schnable.

Before hybridization, arrays were pre-hybridized in a coplin jar at 42° C for 30 - 60 min in a pre-hybridization solution, followed by immersing in water and rising in isopropanol to ensure complete removal of SDS. Arrays were immediately spin-dried in a centrifuge at 1500 rpm in 50 ml tubes each for 5 and 3 min at RT with a piece of dust-free paper on the bottom exchanged between centrifugations.

2.2.2 SCMV arrays

E.coli containing genes of interest were ordered as stab cultures from Arizona BAC/EST resource centre and from the Iowa Schnable Lab, USA. Ten different vectors were utilised: PBluescript SK (-), PBluescript II SK (+), pAD-GAL 4, pAD-GAL 4-2.1, pBK-CMV, pUC19, pT7T3 PAC, pSlip7, pGAD10 and pCMV-Script EX. Plasmid mini preps were conducted using R.E.A.L® Prep 96 Kit (QiaGen AG, Hilden, Germany) according to manufacturer's instructions. Thereafter, plasmids were amplified using the PCR protocol included in Table 3, with primers designed for each vector (Primer Express™ software, version 1.5, Applied Biosystems, Foster City, USA) (Table 4). The quality of PCR products was checked on 1.5 % agarose gels, applying 2 µl of each sample (Figure 5). Quantification of bands was done in GelPro Analyzer software version 3.1 (Media Cybernetics, Inc., Silver Spring, USA) utilising 1 kb DNA ladder (Fermentas GmbH, St. Leon-Rot, Germany), where

the brightest band (2000 bp) was of known concentration of 92 ng (when loading 5 µl of the ladder). Purification and desalting of samples was done in V-shaped 96 well plates by ethanol / acetate precipitation. 130 µl of EtOH / acetate mix (50 ml 3 M NaOAc, pH 5.2 + 125 ml 96 % EtOH) was added to 50 µl of PCR products, mixed well and precipitated over night at -20° C. Next day, plates were centrifuged at 3500 g at 4° C for 45 min. The supernatant was removed with a multi-channel pipette, 200 µl of -20° C cold 70 % EtOH was added to the pellets and spinned down at 3500 g at 4° C for 20 min. The supernatant was removed and pellets were air dried. Furthermore, pellets were dissolved in variable amounts of 50 % dimethyl sulphoxide (DMSO) according to PCR quantification to achieve the final concentration of 420 ng / µl. 5 µl of the samples kept in 96 well plates were transferred to 386 well plates and spotted to Nexterion Slides A+ (SCHOTT JENA^{er} GLAS GmbH, Jena, Germany) using the Qarray mini microarray spotter with 16 pins (Genetix GmbH, Munich, Germany) (Figure 6). Samples were spotted in triplicate in a 9 x 9 pin group design with 16 pin groups on the chip. After spotting, the arrays were air-dried overnight at RT and the DNA was cross-linked to the slides by UV irradiation at 450 mJ (Stratalinker, Stratagene). Slides were stored in an exsiccator in dark containers until usage.

In total SCMV array (Figure 7) contains 878 spots tri-plicated across the slide, and includes 110 wheat controls, 6 maize controls, 302 resistant genes and resistance candidate gene sequences and resistance gene analogues (RGAs) from the China Agricultural University (CAU), Beijing (Prof. Mingliang Xu), as well as 451 differentially expressed genes identified in a previous study (Shi C. et al., 2005), 3 published RGAs (Collins et at., 1998: pic 13, pic 19 duplicated) and 3 exons from the eIF3E barley gene with duplication. Arrays were spotted at the Research Centre Flakkebjerg, Denmark (Dr. G. Dionisio) and utilised for expression profiling experiments.

After fabrication, slides were baked at 80° C for 45 - 60 min in an oven in order to link the spotted DNA to the glass surface, boiled in 1 x SSC for 3-5 min to remove access DNA, and blocked (pre-hybridized). The blocking procedure covered: 1-2 min suspension in 0.5 x SSC at RT, 15 min in Amino Blocking Solution (Nexterion® Block E, Mainz, Germany) at RT, 10-20 sec in 0.1 % SDS at RT, 10-20 sec in diH_2O at RT, 3 min in boiling diH_2O (for PCR-probes spotted arrays) and finally spin-drying in a centrifuge at 200 g in 50 ml tubes separately for each slide for 5 and 3 min at RT with a piece of dust-free paper on the bottom exchanged between centrifugations.

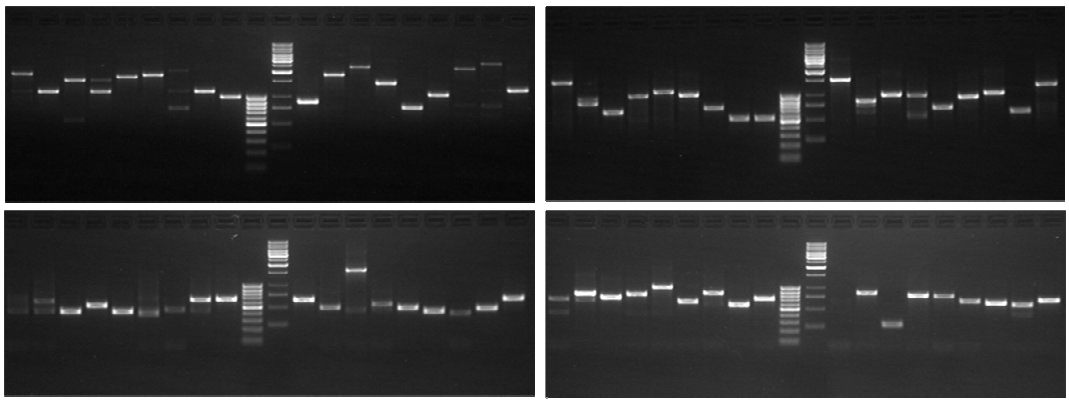

Figure 5. Clones amplification verification on 1.5 % agarose gel before quantification and spotting on SCMV array (1 kb ladder standard, Fermentas)

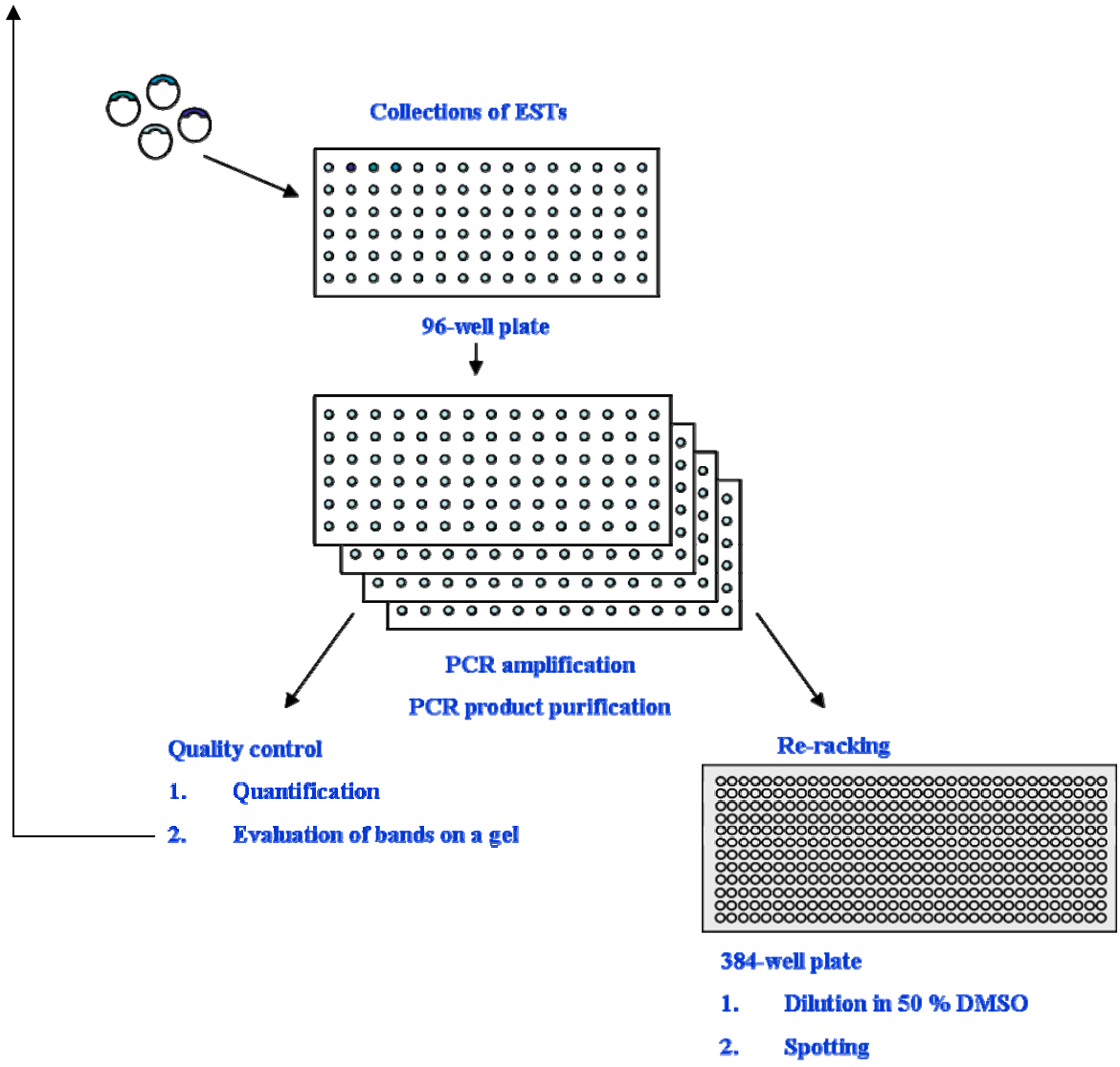

Figure 6. DNA sample preparation for printing cDNA microarrays

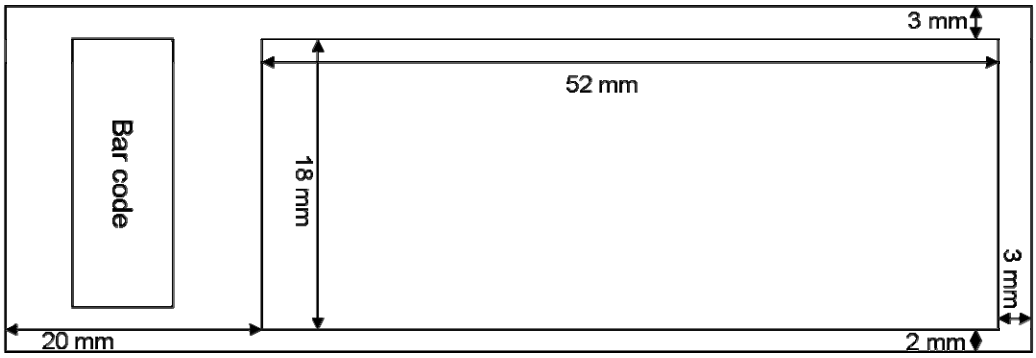

Figure 7. SCMV array layout. Spotted area of the SCMV array: 52 x 18 mm

Table 3. PCR amplification protocol

PCR protocol for vector amplification	Temp. (°C)	Time
denaturation	95° C	2 min
denaturation	95° C	45 sec
primer annealing temp.	56° C	30 sec
extension	72° C	120 sec
go to step 2	42 cycles	
extension	72° C	180 sec
storage	4° C	forever

Table 4. Vectors and their primer sequences for insert amplification

Vector	Forward primer (5' – 3')	Reverse primer (5' – 3')	Temp. (°C)	
			For	Rev
PBluescript SK (-)	GTA AAA CGA CGG CCA GTG	CAG GAA ACA GCT ATG ACC ATG	54.3	55.6
PBluescript II SK (+)	GTA ATA CGA CTC ACT ATA GGG CG	CAA TTA ACC CTC ACT AAA GGG	56.1	54.0
pAD-GAL 4	AAC TTG CGG GGT TTT TCA	TAC CAC TAC AAT GGA TAT GTA TAT AA	56.2	55.8
pAD-GAL 4-2.1	CTA TTC GAT GAT GAA GAT ACC	GAC TAA TAC GAC TCA CTA TAG GGC	54.6	55.4
pBK-CMV	GTA ATA CGA ACT CAC TAT AGG GCG	CAA TTA ACC CTC ACT AAA GGG	56.1	54.0
pUC19	GTA AAA CGA CGG CCA GTG	CAG GAA ACA GCT ATG ACC ATG	54.3	55.6
pT7T3 PAC	GTA AAA CGA CGG CCA GTG	CAG GAA ACA GCT ATG ACC ATG	54.3	55.6
pSlip7	AAT ACG ACT CAC TAT AGG GAG ACA	CAA TTA ACC CTC ACT AAA GGG	55.0	55.6
pGAD 10	CTA TTC GAT GAT GAA GAT ACC	GAC TAA TAC GAC TCA CTA TAG GGC	56.2	55.8
pCMV-Script EX	GTA ATA CGA ACT CAC TAT AGG GCG	CAA TTA ACC CTC ACT AAA GGG	56.1	54.0

2.3 Hybridization design

A total amount of 56 microarray hybridizations (hybrid vs. parent and parent vs. parent) was carried out with four biological dye-swapped replications. The first two replications were performed with a loop-design, where parent 1 (P1) is hybridized to the hybrid on array 1, hybrid to parent 2 (P2) on array 2, and P2 to P1 on array 3, for each triplet. The second two replications were performed with a common pair-wise design, where P1 and P2 are hybridized to the hybrid on two separate arrays (Churchill, 2002; Yang and Speed, 2002). More effort was put on hybrid vs. parent comparisons as this better estimates heterosis.

The design for SCMV experiments was developed in collaboration with Prof. H-P. Piepho (University of Hohenheim, Department of Bioinformatics, Stuttgart, Germany).

The major focus was on the comparison among four near-isogenic genotypes within time points (T1 - T5 and T9), whereas the comparison between six time points (mock control is referred as one time point T9) for a given genotype was of secondary importance. 24 arrays per time point with four biological dye-swapped replications were planned, giving in total 144 hybridizations. The optimal design for six possible pairings of genotypes within a time point was an unresolvable row-column design with six rows corresponding to six slides and two columns corresponding to the two dyes. An example of such a "basic row-column design" is given below:

	Cy3	Cy5
Column / Row +	1	2
1	1	2
2	4	1
3	2	3
4	3	4
5	3	1
6	4	2

2.4 Hybridization conditions

According to hybridization design, a minimum of 25 and maximum of 60 pmols of Cy3 and Cy5 labeled second strand cDNAs were combined, VacuFuge dried (45° C, ~1.5 hours) and dissolved in 5 µl of water and 40 µl of hybridization solution. For heterosis experiment home-made hybridization solution was prepared as indicated below (solutions utilised), while for SCMV experiments Nexterion ready-to-use hybridization solution was utilised (Nexterion® Hyb, Mainz, Germany). Samples were subsequently denatured at 95° C for 3-4

min, quenched on ice for 30 sec and spinned down in a table centrifuge. The Lifter slip (25 x 60 mm) was placed on the array and samples were applied with a pipette, avoiding bubble formation. Arrays were positioned into the hybridization chamber (Genetix BmbH, Munich, Germany) for heterosis experiment and into IHC1 incubation chamber (Quantifoil Instruments, Jena, Germany) for SCMV experiment. 3 ml of 0.5 x SSC was applied to the bottom of the chamber to prevent desiccation. Arrays for heterosis experiment were hybridized in a heating block, while SCMV arrays in an oven, slightly agitating, both overnight at 42° C.

2.5 Post-hybridization washes

2.5.1 Unigene arrays

After hybridization slides were removed from the chamber, Lifter slips discarded in a washing solution 1, slides placed in a slide-holder and washed with post-hybridization solutions. Washings were carried out as followes (Gregersen et al., 2005, with some modifications): 1st washing at 42° C in a washing solution 1 (2 x SSC, 0.1 % SDS) for 10 min, 2nd washing 5 min in washing solution 2 (0.2 % x SSC, 0.1 % SDS) at 42° C and in the same solution for another 5 min at RT, 3rd washing in washing solution 3 (0.1 x SSC) at RT three times for 5 min, and 4th washing in washing solution 4 (0.01 x SSC) in 50 ml tubes per slide for 5-10 sec. Washed slides were immediately spin-dried in the centrifuge at 1500 rpm in 50 ml tubes for 5 and 3 min at RT with a piece of dust-free paper on the bottom and kept covered with aluminium foil to avoid light exposure.

2.5.2 SCMV arrays

After hybridization slides were washed using 1 x SSC, 0.2 % SDS washing solution 1, at 55° C for 10 min, 0.2 x SSC, 0.1 % SDS at 55° C for 10 min, 0.1 x SSC at RT for 10 min and rinsed in 80 % ethanol, subsequently spin-dried and stored as described in 2.6.1.
The washing procedure was optimized for Nexterion slides (http://www.us.schott.com/nexterion/english/download/protocol_slide_aplus_us.pdf).

2.6 Scanning

Microarrays were scanned using arrayWoRx microarray scanner (BioChipReader, Applied Precision, Washington, USA) for unigene arrays and Gene TAC UC 4 x 4 microarray scanner (GeneMachines $_{TM}$, Genomic Solutions, Inc., USA) for SCMV arrays, generating 16-bit greyscale TIFF files for each channel separately, as well as a composite file (Figure 8). Quantification based on TIFF files was done in Array Vision, version 8.0 (Imaging Research Inc., St. Catharines, Canada), with spot grids aligned manually for each slide and local background calculated from the corners between spots.

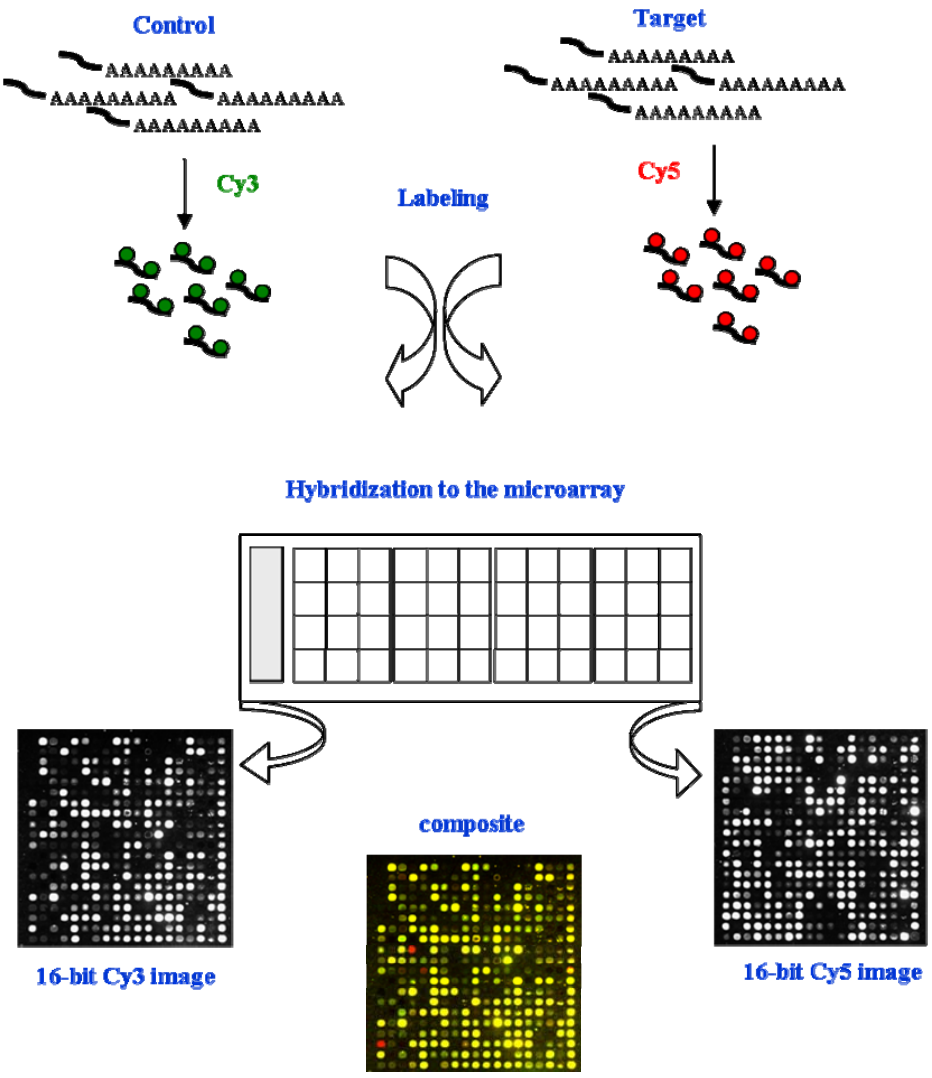

Figure 8. cDNA labelling, hybridization and data acquisition

Solutions utilized:

Hybridization solution for unigene microarray hybridization
50 % formamide (deionized)
3 x SSC
5 x Denhardts
0.1 % SDS
Optionally: 5 % dextran sulphate
100 µg / ml salmon sperm DNA
20 µg / ml poly A DNA

2.7 QRT-PCR experiments

2.7.1 Plant materials

Apical meristems from four biological replications for inbred lines 250, 301 and hybrids 005x250, 250x002, and 301x005 were used for heterosis qRT experiment. Two biological replications were used from the material grown in Denmark for microarray experiments and two new replications were grown in Freising (5 genotypes, 10 plants per genotype, completely randomized blocks).

For the SCMV qRT candidate gene validation, leaf tissue of genotypes F7 $^{RR/RR}$, F7 $^{SS/RR}$ and F7 $^{RR/SS}$ with four biological replications utilised in SCMV microarray study was used.

2.7.2 RNA isolation

Total RNA was isolated using the TRIzol reagent (Invitrogen GmbH, Karlsruhe, Germany). To 100 mg of grinded sample stored at -80° C in 2 ml Eppendorf tubes 1 ml of TRIzol reagent was added and the mixture was incubated for at least 5 min at RT gently agitating. Afterwards, 0.2 ml of chloroform was applied and shaken strongly for 15 sec, subsequently centrifuged at 1200 g at 2-8° C for 15 min. The liquid separated in three phases: the upper aqueous phase contained RNA, the interphase contained gDNA and into lowest organic phase contained phenol, chloroform and organic molecules. The colourless upper aqueous phase was transferred into fresh 2 ml Eppendorf tubes, while paying attention to not

contaminate the RNA with material from the other phases. 0.5 ml of 100 % isopropanol was added, gently mixed and incubated for 10 min at RT, thereafter centrifuged at 1200 g for 10 min and the supernatant discarded. The RNA pellets became visible at this step. Pellets were purified with 1 ml of 75 % EtOH, vortexed gently and centrifuged at max. g for 5 min. EtOH was discarded and the step was repeated. After purification, pellets were air-dried at RT for 10 min, thereafter resuspended in 50-100 µl of 0.1 % DEPC-water (depending on the size of the pellet). Samples were incubated in a water-bath at 55-60° C for 10 min to resuspend the pellet. Samples were divided into aliquots for quantification (1 µl RNA in 99 µl of water for spectrophotometry and 2 µl RNA in 8 µl of formamide loading buffer for elecrophoresis). The rest of the samples were deep-frozen at -80° C. Quantification of samples was done using a spectrophotometer (Genesys 10 Bio, Thermo electron corporation, Madison, USA), where the absorption at 260 and 280 nm and the 260 / 280 ratio were measured. Acceptable values for RNA purity were the range of 1.5 - 1.8.

RNA was loaded on 1.2 % formaldehyde agarose gels for quality control, after previous sample denaturation at 65° C for 10 min and cooling on ice. The probes (2 µl RNA + 8 µl formamide loading buffer) were run on the gel at 70 V for 2 hours under the hood. Two discrete bands of 28S and 18S RNA should become visible (Figure 9).

RNA clean-up of stock RNA samples was performed on RNeasy mini kit columns (QiaGen AG, Hilden, Germany) following manufacturer's instructions, with previous DNA digestion. The digestion for heterosis qRT samples preparation was performed using RNase free DNase (1500 Kunitz units) in microcentrifuge tubes (Qiagen AG, Hilden, Germany). 50 ng of RNA was mixed with 10 µl of RDD buffer, 2.5 µl of DNase, and filled up to 100 µl with RNase free water and incubated at 20-25° C for 10 min. For SCMV samples 40 ng of isolated RNA was mixed with 10 µl of 10 x reaction buffer containing $MgCl_2$, 40 µl of RNase free DNase I (5000 units) (peqDOLG DNase, Peqlab, Germany), filled up to 100 µl with RNase free water and incubated at 37° C for 30 min.

After purification, samples were quantified spectrophotometrically (1 µl RNA in 99 µl of water) (Genesys 10 Bio, Thermo electron corporation, Madison, USA).

Sequence specific primers for reference and target genes were designed using Primer Express™ software, version 1.5 (Applied Biosystems, Foster City, USA) for both heterosis and SCMV experiments (Tables 5 and 6). Primers were tested for optimal annealing temperatures and quality by gradient PCR before qRT-PCR analyses (Figure 10).

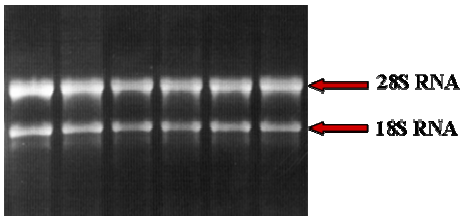

Figure 9. 28S and 18S RNA subunits on a 1.9 % formaldehyde agarose gel (apical meristem)

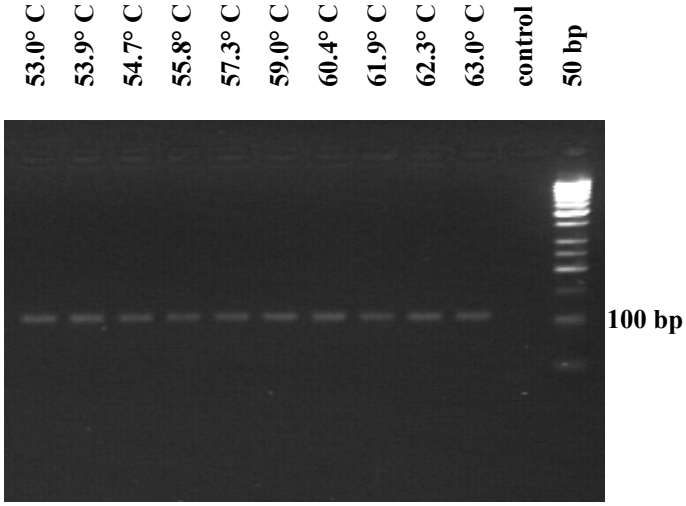

Figure 10. Example of a gradient PCR for the S-adenosylmethionine synthetase 1 gene

Table 5. Primer sequences for qRT-PCR heterosis experiments. Sequence specific primers for reference (actin) and three target genes

Gene name	Primer sequence (5' - 3')	Annealing temp.
Maize actin 1	For : TCC TGA CAC TGA AGT ACC CGA TTG Rev: CGT TGT AGA AGG TGT GAT GCC AGT T	58° C
Putative defensin	For : GCA AGC GGA TCT GCT AGC T Rev: CAG ACG GAC ACG CAC GTA C	58° C
Geranyl-geranyl reductase	For : CAT CGA TAC AAA CAG GCA GCA Rev: AAT TTA GGC CAA CAT GCG TG	58° C
Gibberellin-stimulated transcript 1 like protein	For : GAC TCT GGA TCG GCG GAT Rev : CCC TCT CAC TCT GGT GCA CA	58° C

Table 6. Primer sequences for qRT-PCR SCMV experiments. Sequence specific primers for reference (actin) and five target genes

Gene name	Primer sequence (5' - 3')	Annealing temp.
Maize actin 1	For : TCC TGA CAC TGA AGT ACC CGA TTG Rev: CGT TGT AGA AGG TGT GAT GCC AGT T	60.5° C
26S ribosomal RNA gene	For : CAT TCA ATC GGT AGG AGC GAC Rev: GGT CTT CAA CGA GGA ATG CC	60.5° C
Metallothionein-like protein	For : ACT CGG CCC ACA CAG CA Rev: GAG ATG TTG GCG CCG TG	60.5° C
S-adenosylmethionine synthetase 1	For : CCT ATC GGT GTT CGT GGA CA Rev : TGA TCA TGC CGG GCC T	60.5° C
14-3-3-like protein GF14-6	For : GGG AGC CCC CAA ATT TTA CT Rev: AGT GTT TGC TGC TGT CGA ATG	60.5° C

2.7.3 QRT-PCR

Quantitative RT-PCR experiments were conducted with One-Step QuantiTect SYBR® Green RT-PCR Kit (Qiagen AG, Hilden, Germany) on a 7300 RT PCR System (Applied Biosystems, Foster City, USA) for heterosis and ABI PRISM™ 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) for SCMV experiment under the following conditions: 50° C for 30 min, 95° C for 15 min and 45 cycles of 94° C for 30 sec, 58° C for 15 sec, and 72° C for 30 sec in total volumes of 25 µl reaction. Four biological and three technical replications were used for every gene in order to precisely quantify the transcript abundance (Table 7). 100 ng per 3 µl RNA was used for target and reference genes, and 200 ng per 3 µl for dilution series.

To identify primer-dimers and unspecific PCR products, dissociation curve analyses were implemented. Dissociation curves were prepared with following serial dilutions: 100 %, 50 %, 10 %, 5 %, 1 %, 0.5 %, 0.1 % and water (NTC). An endogenous reference sequence was deduced from the maize actin gene (MAc1) (EMBL-EBI Accession No. J01238) using primers Act for and Act rev (Tables 5 and 6).

Solutions used:

RT-PCR mix (reference and target genes)	1 x (µl)
RT-mix	0.25
SYBR Green	12.5
Forward primer (5' – 3')	1.0
Reverse primer (5' – 3')	1.0
RNA	3.0
Water	7.25
Total	25.0

Formamide – dye-mix	Amount (µl)
formamide	1 ml
0.5 M EDTA	20 µl
dye-mix	20 µl
Dye mix: 50 mg of bromophenol blue + 50 mg of xylencyanol diluted in 1 ml of 0.1 % DEPC water	

Formamide loading buffer	In 1 ml
5 x MOPS	200 µl
Formaldehyde	175 µl
Formamide	500 µl
Formamide – dye-mix	100 µl
EtBr (10 µg / µl)	25 µl

5 x electrophoresis buffer		End concentration in 1 l
400 ml	0.5 M MOPS (Sigma)	0.2 M MOPS
16.6 ml	3 M NaAc (Sigma)	50 mM NaAc
10 ml	0.5 M EDTA (Sigma)	5 mM EDTA
537.4 ml	water	

Table 7. qRT-PCR sample sheet. Target gene: gene of interest, reference (ref) gene: Mac1 (Maize Actin 1), NTC: water, target sample 1-4: first genotype to be compared with four biological replications, target sample 5-8: second genotype to be compared with four biological replications, ref sample 1-4: Mac1 for first genotype with four biological replications, ref sample 4-8: Mac1 for second genotype with four biological replication

	1	2	3	4	5	6	7	8	9	10	11	12	
A	Target gene 100 %	Target gene 100 %	Target gene 100 %	Ref gene 100 %	Ref gene 100 %	Ref gene 100 %	Target sample 1	Target sample 1	Target sample 1	Ref sample 1	Ref sample 1	Ref sample 1	A
B	Target gene 50 %	Target gene 50 %	Target gene 50 %	Ref gene 50 %	Ref gene 50 %	Ref gene 50 %	Target sample 2	Target sample 2	Target sample 2	Ref sample 2	Ref sample 2	Ref sample 2	B
C	Target gene 10 %	Target gene 10 %	Target gene 10 %	Ref gene 10 %	Ref gene 10 %	Ref gene 10 %	Target sample 3	Target sample 3	Target sample 3	Ref sample 3	Ref sample 3	Ref sample 3	C
D	Target gene 5 %	Target gene 5 %	Target gene 5 %	Ref gene 5 %	Ref gene 5 %	Ref gene 5 %	Target sample 4	Target sample 4	Target sample 4	Ref sample 4	Ref sample 4	Ref sample 4	D
E	Target gene 1 %	Target gene 1 %	Target gene 1 %	Ref gene 1 %	Ref gene 1 %	Ref gene 1 %	Target sample 5	Target sample 5	Target sample 5	Ref sample 5	Ref sample 5	Ref sample 5	E
F	Target gene 0.5 %	Target gene 0.5 %	Target gene 0.5 %	Ref gene 0.5 %	Ref gene 0.5 %	Ref gene 0.5 %	Target sample 6	Target sample 6	Target sample 6	Ref sample 6	Ref sample 6	Ref sample 6	F
G	Target gene 0.1 %	Target gene 0.1 %	Target gene 0.1 %	Ref gene 0.1 %	Ref gene 0.1 %	Ref gene 0.1 %	Target sample 7	Target sample 7	Target sample 7	Ref sample 7	Ref sample 7	Ref sample 7	G
H	NTC	NTC	NTC	NTC	NTC	NTC	Target sample 8	Target sample 8	Target sample 8	Ref sample 8	Ref sample 8	Ref sample 8	H
	1	2	3	4	5	6	7	8	9	10	11	12	

2.8 Statistics

The morphological data (PHT, NOI and INT) were analysed in PLABSTAT version 2F (a computer software for statistical analysis of plant breeding experiments), developed at the University of Hohenheim (http://www.uni-hohenheim.de/~ipspwww/soft.html). Genotype means were computed per location based on a split-plot analysis of variance. Subsequently, two-factorial ANOVA with genotypes (G) and locations (O) as random factors was applied to the means per location and genotype. Estimates for heritabilities on a plot base, variance components, and coefficients of correlation were calculated.

Mid-parent heterosis for six triplets was calculated from absolute values as:

$MPH = \frac{(F_1 - MP)}{MP} \times 100$, where F1 is the mean of hybrid performance, MP = (P1 + P2) / 2 is the average of inbred parent means.

High-parent heterosis was calculated as following:

$HPH = \frac{(F_1 - HP)}{HP} \times 100$, where HP is the mean of the higher parent (Betrán et al., 2003).

Raw intensity values from the scans with background correction from the corners between spots were utilised for heterosis expression profiling data analysis. Locally weighted scatterplot smoothing regression (Lowess) algorithm was performed to normalize data regarding scale and dye effects and centering was accomplished to normalize between arrays (Stekel, 2003). A mixed model was used to model log-data for each spot. Genotype, dye, season, replicate and genotype*season interaction were treated as fixed, the array being the only random effect. Thus the recovery of inter-array information was possible. Estimates for the genotypes as well as estimates for hybrid vs. parent contrasts and estimates of mid-parent heterosis were determined. If the genotype*season interaction was significant (after p-value adjustment) the main effects of genotype and season were removed from the model and the above-mentioned estimates were calculated separately for each season. P-values of effects or linear contrasts of effects were adjusted with the FDR-method (5 %) (Benjamini and Hochberg, 1995). All calculations were performed with the SAS System for Windows, Version 9.1.

Due to the fact that plants for expression profiling experiments were grown in two seasons only genes with no genotype*season interaction and with p values for estimates ≤ 0.05 were chosen for further quantitative analyses as significantly differentially expressed.

Blast analysis was performed in TIGR Unique Gene Indices (http://tigrblast.tigr.org/tgi/), from *Arabidopsis thaliana*, barley (*Hordeum vulgare*), maize (*Zea mays*), rice (*Oryza sativa*), rye (*Secale cereale*) and wheat (*Triticum aestivum*), with a cut off e-value of 10 (Ros et al., 2004). A self-organizing tree algorithm (SOTA) for gene clustering was employed according to TIGR MultiExperimentViewer software (Version 3.1).

Based on the estimates for each genotype it was possible to calculate the degree of dominance with regard to expression profiles for all differentially expressed genes on the unigene microarray. The dominance / additivity ratios were calculated as follows: d / a = (hybrid - 0.5 * (parent 1 + parent 2)) / abs (parent 1 - parent 2) (as in Tanksley, 1993 and Stuber, 1987), where the d / a ratio intervals from -0.2 to 0.2 reflected additive gene action, 0.2 to 0.8 and -0.2 to -0.8 partial dominance, 0.8 to 1.2 and -0.8 to -1.2 dominance and > 1.2 and < -1.2 overdominance.

For the SCMV resistance expression profiling study, raw intensity and background values generated by Array Vision were used for data analysis. The main interest was to determine the expression patterns of pairwise contrasts between genotypes at the same time point. Contrasts of a genotype at two different time points were of secondary interest. After background correction Lowess regression was performed to adjust for differences within an array.
The following linear mixed model was fitted:

$$y_{ijkl} = g_i + t_j + a_k + d_l + (g*t)_{ij} + (g*d)_{il} + e_{ijkl},$$

where y_{ijkl} is the log2-signal intensity, g_i is a fixed effect for genotype, t_j is a fixed effect for the time point, a_k is a random effect for the array and d_l is a fixed effect for the dye. Furthermore we considered interactions $(g*t)_{ij}$ and $(g*d)_{il}$ between genotype and time point and genotype and dye, respectively. The calculations were performed with the SAS System for Windows, Version 9.1.
Pair-wise contrasts between different genotype*time combinations were estimated, considering only contrasts between genotypes within one time point and contrasts of one genotype at different time points. The corresponding FDR adjusted p-values and fold changes were determined. Least square means of genotype*time were calculated, i.e. the value of a certain genotype at a specific time point averaged over the other effects. The degrees of freedom for the tests were calculated according to the containment method.

Similarly to heterosis expression profiling, blast analysis in TIGR Unique Gene Indices was performed in order to identify the putative function of unknown sequences from *Arabidopsis thaliana*, barley, maize, rice, rye and wheat.

Quantitative Real-time PCR efficiencies ($E=10^{(-1/slope)}-1$), were derived from calibration data of serially diluted RNA and relative expression rates of the target genes were calculated as follows:

$rel.expression = \frac{(1+E_{target})^{\Delta Ct_{target}}}{(1+E_{ref})^{\Delta Ct_{ref}}}$, where E_{target} is the PCR efficiency for the target gene and E_{ref} is the PCR efficiency for the endogenous reference. ΔCt_{target} and ΔCt_{ref} values were determined as described by Dilger, (2003). The influence of genotypes on the expression of the endogenous reference gene was evaluated by one-way ANOVA ($p < 0.01$).

3 RESULTS

3.1 Heterosis

3.1.1 Morphology

In the phenotypic survey high heritabilities, ranging from ~84 to 97 % for PHT, 94 to 99 % for NOI and 71 to 97 % for INT3 - 5, 8 and 9, were detected. Low heritability values for INT1, 2, 6, and 7 and for width of internodes discarded these traits from further analyses.

Hybrid PHT was significantly higher ($p < 0.05$) in the field as compared to the greenhouse experiments, whereas inbred lines were substantially lower in the field as compared to the greenhouse (Figures 11 and 12). Both hybrids and the inbred lines displayed significantly more internodes in the field as compared to those grown in the greenhouse, but length of internodes (INT3, 4, 5, 8, 9) showed a tendency to decrease with internode number in inbred lines in both environments. Hybrids had longer internodes in the field as compared to the greenhouse, except of INT9 (Table 8).

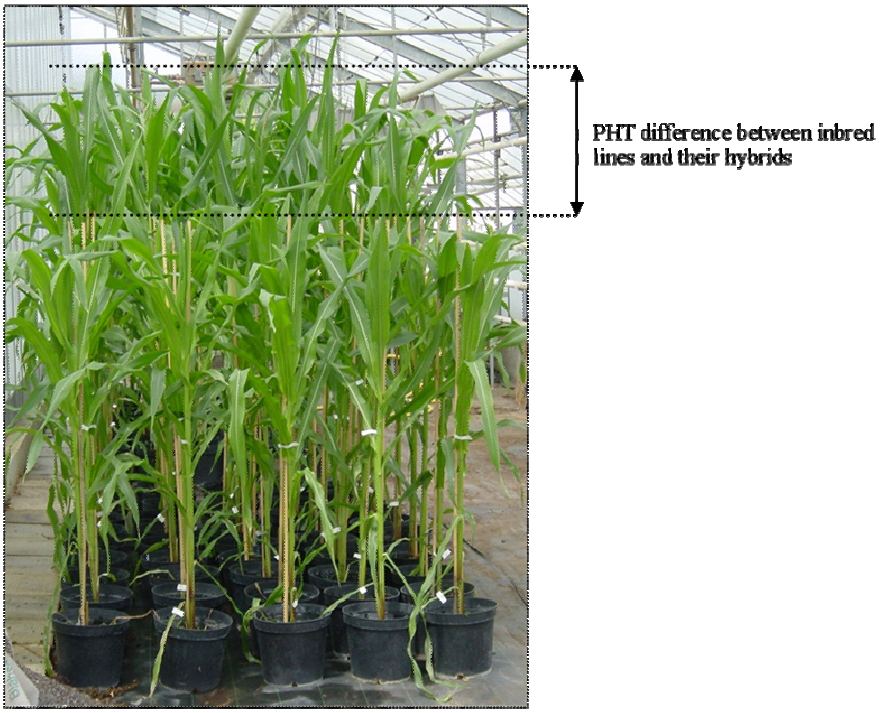

Figure 11. The difference in PHT of inbreds and their hybrids in optimal greenhouse conditions

Figure 12. The difference in PHT of inbreds and their hybrids in the field

The average mid-parent heterosis (MPH) for PHT in the field was 48.5 %, and 28.2 % in the greenhouse. Average MPH for NOI in the field was 7.8 %, and 2.5 % in the greenhouse. For internode length the average MPH was highest for INT9, with 150.3 % in the field and 59.7 % in the greenhouse, followed by INT8, with 100.4 % in the field and 44.6 % in the greenhouse. The same pattern was observed for every single triplet except of TRIP 2 for INT8 in the greenhouse. For INT3 - 5 the MPH value did not exceed 31.7 % in the field and 29.1 % in the greenhouse (Table 8, Figure 13). Significant MPH estimates ($p < 0.01$) for greenhouse were found for PHT, NOI, INT4 and INT8, for INT5 at $p < 0.05$. In field experiments MPH for PHT was significant at the level of $p < 0.05$ and for NOI on $p < 0.1$.

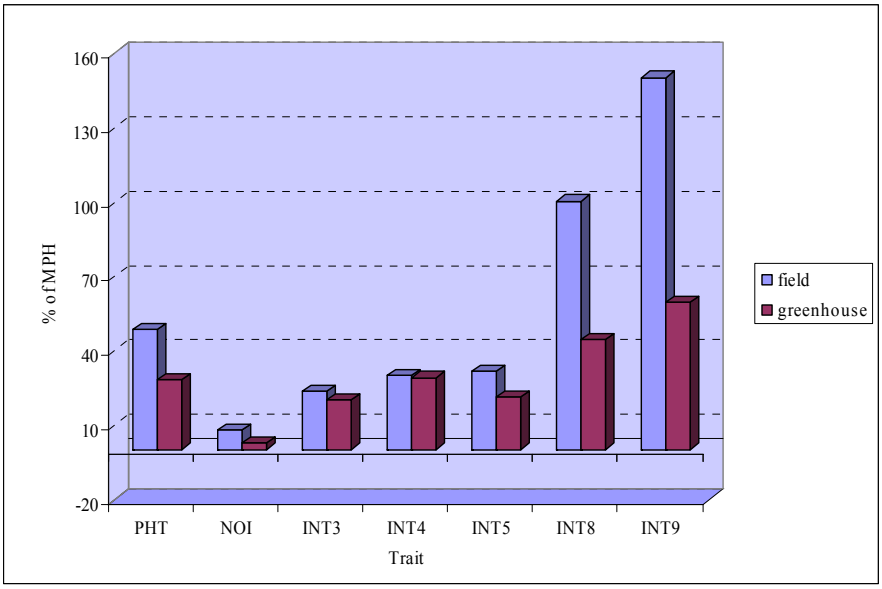

Figure 13. Mid-parent heterosis (average 2004 / 2005)

Average HPH for PHT was more than twice as high in the field (42.2 %) as compared to the greenhouse (16.5 %), while HPH for NOI was 0.6 % for field and negative for the greenhouse -5.8 %. Average HPH for length of internodes was highest for INT9 in the field (89.9 %) and for INT8 (27.2 %) in the greenhouse (Table 8, Figure 14). Significant estimates for HPH (greenhouse) ($p < 0.01$) were obtained for PHT, NOI, INT4, at $p < 0.05$ for INT5 and for INT8, and at $p < 0.1$ for INT3. In the field HPH was only significant for PHT ($p < 0.05$). The interpretation of the significance of results should be, however, done with care, since tests on MPH and HPH were performed on only six triplets.

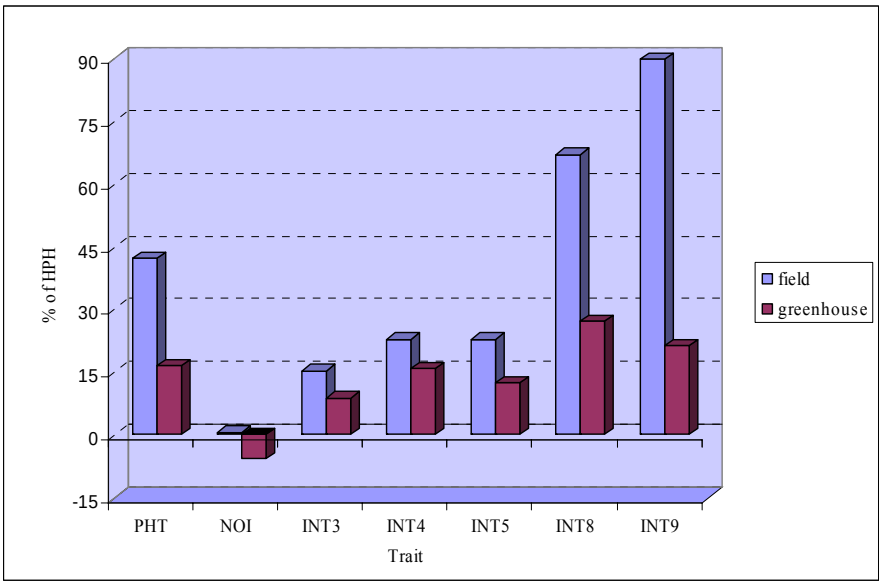

Figure 14. High-parent heterosis (average 2004 / 2005)

Consistent correlations for hybrids in the field (Table 9) were found for PHT with NOI (0.825, $p < 0.05$) and for PHT with INT8 and INT9, respectively (0.881, $p < 0.05$; 0.946, $p < 0.01$). In the greenhouse, PHT was also significantly correlated with INT8 (0.922, $p < 0.01$) and INT9 (0.862, $p < 0.05$). In addition, consistent correlations were found between NOI and INT3, 5 and 9 in the field, and between selected internodes in the field as well as in the greenhouse (field: INT3 and INT8, INT4 and INT5, INT8 and INT9; greenhouse: INT 3 and INT5, INT4 and INT5).

Table 8. Morphological traits: plant height (PHT), number of internodes (NOI) and length of selected internodes (INT). Mean values were calculated for n = 32 plants in the field and n = 40 plants in the greenhouse in both years for plant height (PHT), number of internodes (NOI) and length of internodes (INT), in independent field and greenhouse experiments in 2004 and 2005 ± SE. P1: parent 1, P2: parent 2, F1: hybrid. MPH: mid-parent heterosis, HPH: high-parent heterosis, TRIP 1: 002_002x301_301, TRIP 2: 005_005x250_250, TRIP 3: 250_250x002_002, TRIP 4: 301_301x005_005, TRIP 5: 002_002x005_005, TRIP 6: 250_250x301_301)

	Field						Greenhouse						Average field ± SE	Average greenhouse ± SE
	TRIP 1	**TRIP 2**	**TRIP 3**	**TRIP 4**	**TRIP 5**	**TRIP 6**	**TRIP 1**	**TRIP 2**	**TRIP 3**	**TRIP 4**	**TRIP 5**	**TRIP 6**		
PHT														
P1	158.87	145.53	161.50	173.14	158.87	161.50	148.81	155.22	182.79	212.71	148.81	182.79	159.7 ± 3.22	174.9 ± 4.88
P2	173.14	161.50	158.87	145.53	145.53	173.14	212.71	182.79	148.81	155.22	155.22	212.71		
F1	239.11	239.58	238.68	247.63	212.42	246.00	238.90	224.97	221.96	242.96	193.99	218.77	237.2 ± 3.12	223.6 ± 6.82
MPH %	44.03	56.06	49.00	55.41	39.57	47.02	32.16	33.11	33.87	32.07	27.61	10.63	48.5	28.2
HPH %	38.10	48.35	47.79	43.02	33.71	42.08	12.31	23.08	21.43	14.22	24.98	2.85	42.2	16.5
NOI														
P1	10.82	11.37	13.73	13.27	10.82	13.73	10.14	11.05	13.86	12.70	10.14	13.86	12.3 ± 0.21	11.9 ± 0.12
P2	13.27	13.73	10.82	11.37	11.37	13.27	12.70	13.86	10.14	11.05	11.05	12.70		
F1	12.86	14.35	13.57	13.19	11.29	14.33	11.69	13.43	12.27	12.19	10.52	13.35	13.3 ± 0.15	12.2 ± 0.09
MPH %	6.77	14.34	10.55	7.06	1.76	6.15	2.36	7.83	2.25	2.65	-0.71	0.53	7.8	2.5
HPH %	-3.09	4.52	-1.17	-0.60	-0.70	4.37	-7.95	-3.10	-11.47	-4.02	-4.80	-3.68	0.6	-5.8
INT3														
P1	19.13	15.46	14.76	16.80	19.13	14.76	18.09	14.15	13.93	19.98	18.09	13.93	16.5 ± 0.37	16.5 ± 0.94
P2	16.80	14.76	19.13	15.46	15.46	16.80	19.98	13.93	18.09	14.15	14.15	19.98		
F1	20.51	18.62	19.10	21.00	22.58	20.52	23.36	17.44	19.77	19.92	21.11	17.51	20.4 ± 0.60	19.9 ± 0.66
MPH %	14.16	23.23	12.71	30.19	30.56	30.04	22.72	24.22	23.49	16.73	30.96	3.27	23.5	20.2
HPH %	7.21	20.44	-0.17	25.00	18.03	22.14	16.92	23.25	9.29	-0.30	16.69	-12.36	15.4	8.9
INT4														
P1	17.79	14.48	14.82	16.08	17.79	14.82	16.33	12.32	14.55	19.21	16.33	14.55	15.8 ± 0.40	15.6 ± 0.70
P2	16.08	14.82	17.79	14.48	14.48	16.08	19.21	14.55	16.33	12.32	12.32	19.21		
F1	21.37	19.27	20.74	20.05	21.24	20.36	25.15	18.99	20.57	19.77	18.30	17.79	20.5 ± 0.53	20.1 ± 0.64

MPH %	26.19	31.54	27.20	31.21	31.64	31.78	41.53	41.35	33.23	25.40	27.75	5.39	30.1	29.1
HPH %	20.12	30.03	16.58	24.69	19.39	26.62	30.92	30.52	25.96	2.92	12.06	-7.39	22.9	15.8
INT5														
P1	18.63	13.94	15.07	15.27	18.63	15.07	15.79	13.02	15.24	17.67	15.79	15.24	15.73 ± 0.40	15.43 ± 0.61
P2	15.27	15.07	18.63	13.94	13.94	15.27	17.67	15.24	15.79	13.02	13.02	17.67		
F1	21.73	18.80	21.00	20.40	22.07	20.09	21.83	18.24	19.13	17.34	18.74	16.55	20.68 ± 0.45	18.64 ± 0.70
MPH %	28.14	29.61	24.63	39.68	35.52	32.43	30.48	29.09	23.30	13.00	30.09	0.58	31.7	21.1
HPH %	16.59	24.75	12.72	33.60	18.46	31.57	23.54	19.69	21.15	-1.87	18.68	-6.34	22.95	12.5
INT8														
P1	6.34	7.81	12.32	11.57	6.34	12.32	9.36	12.31	15.25	14.49	9.36	15.25	9.51 ± 0.82	11.83 ± 0.78
P2	11.57	12.32	6.34	7.81	7.81	11.57	14.49	15.25	9.36	12.31	12.31	14.49		
F1	20.54	20.94	21.05	20.26	12.17	18.91	20.20	18.96	18.86	19.98	15.47	17.14	18.98 ± 0.84	18.44 ± 0.75
MPH %	129.37	108.04	125.62	109.08	72.01	58.31	69.39	37.59	53.27	49.10	42.78	15.27	100.4	44.6
HPH %	77.53	69.97	70.86	75.11	55.83	53.49	39.41	24.33	23.67	37.89	25.67	12.39	67.13	27.23
INT9														
P1	2.70	4.59	8.48	8.7	2.70	8.48	4.23	7.74	14.09	13.19	4.23	14.09	6.12 ± 0.70	9.82 ± 0.65
P2	8.70	8.48	2.70	4.59	4.59	8.70	13.19	14.09	4.23	7.74	7.74	13.19		
F1	14.71	17.84	16.53	17.49	7.96	16.6	15.61	17.50	16.21	19.59	7.99	16.79	15.2 ± 0.84	15.62 ± 0.68
MPH %	158.07	172.99	195.71	163.21	118.38	93.25	78.30	60.33	76.97	87.20	32.50	23.09	150.3	59.7
HPH %	69.08	110.38	94.93	101.03	73.42	90.80	18.35	24.20	15.05	48.52	3.23	19.16	89.94	21.42

Table 9. Correlations between plant height (PHT) and number (NOI) and length of internodes (INT) (for hybrids) GH: greenhouse, F: field, * significant at $p < 0.05$, ** significant at $p < 0.01$

GH / F	PHT	NOI	INT3	INT4	INT5	INT8	INT9
PHT		0.398	0.132	0.573	0.185	0.922**	0.862*
NOI	0.825*		-0.785	-0.245	-0.469	0.328	0.761
INT3	-0.584	0.819*		0.783	0.832*	0.212	-0.373
INT4	-0.502	-0.713	0.594		0.897*	0.677	0.149
INT5	-0.588	0.844*	0.694	0.968**		0.366	-0.244
INT8	0.881*	0.790	-0.830*	-0.458	-0.549		0.788
INT9	0.946**	0.908*	-0.790	-0.682	-0.753	0.934**	

3.1.2 Expression profiling

3.1.2.1 Hybridization results

The expression profiling experiment was carried out in order to identify candidate genes related to plant height heterosis. Due to the fact that lowermost internodes are being formed earliest during plant development and that the highest levels for PHT heterosis were found for internodes INT8 and INT9, showing close correlations to PHT, it was decided to use the apical meristem at the terminal spikelet stage in our analysis (Figure 15). At this stage, all internodes have already been formed. Plants for expression profiling experiments were grown under controlled greenhouse conditions, to avoid biotic or abiotic stress that might affect gene expression levels.

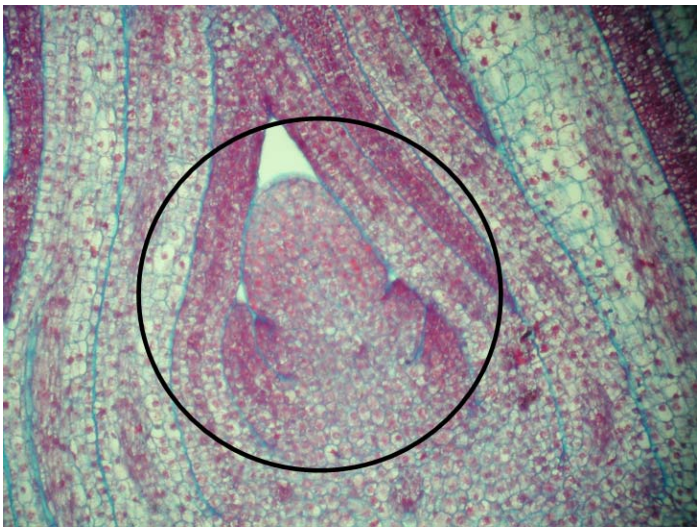

Figure 15. Maize meristem at developmental stage 14-15 (BBCH). Sample was fixed using the FAA [formaldehyde-glacial acetic acid-ethanol] method and stained with alcian blue and safranin dyes. The apical meristem (*circle*) was used for RNA isolation. The fixation in FAA covered: dehydration in graded TBA series, paraffin embedding, paraffin removal in Histoclear, rehydration in graded IPA series, staining in safranin and alcian blue, mounting in Pertex

Eight possible hybrid versus inbred comparisons within four triplet combinations were considered in this experiment: 002 vs. 002x301 and 301 vs. 002x301 for TRIP 002_002x301_301, 005 vs. 005x250 and 250 vs. 002x250 for TRIP 005_005x250_250, 250 vs. 250x002 and 002 vs. 250x002 for TRIP 250_250x002_002, and 301 vs. 301x005 and 005 vs. 301x005 for TRIP 301_301x005_005. Out of 12032 genes on the array from 9271 to 9506 were found without genotype*environment interaction for individual hybrid-inbred comparisons (Figure 16). Within this group, up to 99 genes were found to be significantly differentially expressed for particular comparisons (Table 10). The majority of significantly differentially expressed genes were found for comparison 301 vs. 301x005 (1.07 %), followed by 250 vs. 250x002 (0.87 %), 301 vs. 002x301 (0.86 %), 005 vs. 005x250 (0.68 %), 250 vs.

005x250 (0.62 %), 005 vs. 301x005 (0.43 %) and finally for comparison 002 vs. 250x002 (0.06 %). For comparison 002 vs. 002x301 no significantly differentially genes were found. Most of genes were found for TRIP 301_301x005_005 (0.74 %), followed by TRIP 005_005x250_250 (0.65 %), TRIP 250_250x002_002 (0.47 %), and finally TRIP 002_002x301_301 (0.42 %). Within all hybrid vs. inbred comparisons, altogether 433 genes were significantly differentially expressed at a significance level of $p \leq 0.05$ (Table 8, Annex: Tables 1 - 7), which accounted for ~ 0.57 % of all 75515 gene-triplet combinations considered in individual experiments without significant genotype*environment interaction. Across all comparisons about 75 % of genes were up-regulated in a hybrid, with the highest value for comparison 005 vs. 301x005 (78.0 %) and the lowest for comparison 002 vs. 250x002 (66.7 %). 93.1 % of the 433 differentially expressed genes showed less than two-fold differential expression between hybrids vs. parental lines, 3.9 % between two- and three-fold, 2.6 % between three- and four-fold, and 0.2 % more than four-fold altered expression levels. For lower than two-fold differential expression, most differentially expressed genes were found in comparison 301 vs. 301x 005 (130 genes), between two- and three-fold in comparison 005 vs. 301x005 (7 genes), between three- and four-fold in comparison 250 vs. 250x002 (5 genes) and 1 gene showed more than four-fold altered expression levels for comparison 301 vs. 301x005.

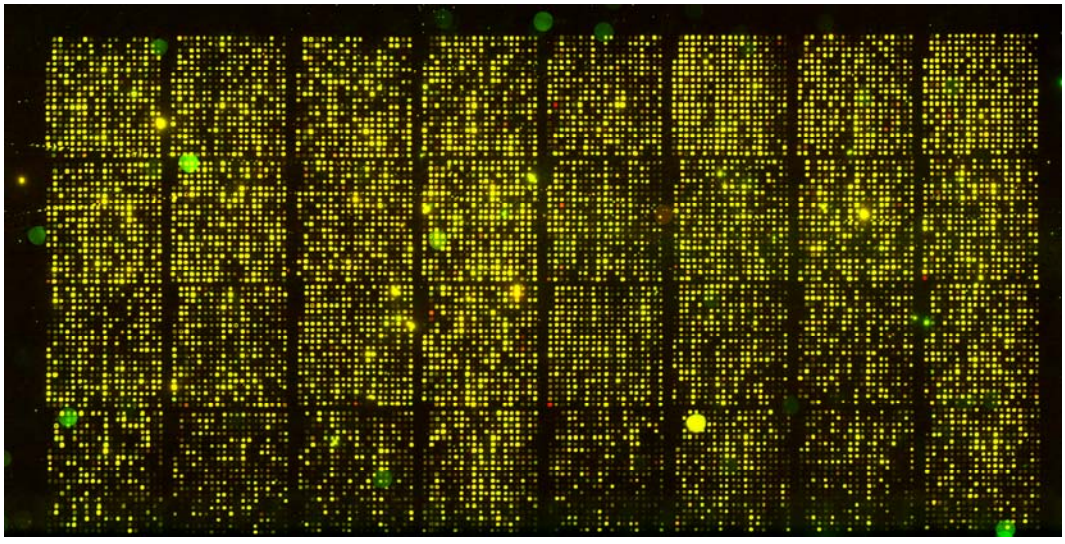

Figure 16. Hybridized unigene microarray

Table 10. Significantly differentially expressed genes from microarray experiments (without genotype*environment interaction). The unigene microarray contained 12032 ESTs. Genes with significant genotype*environment interaction were excluded from the analysis

TRIP	Hybrid genotype vs. inbred genotype	Genes without interaction	Genes significantly differentially expressed ($p \leq 0.05$)	Genes sign. diff. exp. up-regulated (in a hybrid)	Genes sign. diff. exp. up-regulated [%]
1	002x301 vs. 002	9 506	0	0	0
	002x301 vs. 301	9 271	80	61	76.2
2	005x250 vs. 005	9 504	65	47	72.3
	005x250 vs. 250	9 495	59	44	74.6
3	250x002 vs. 250	9 496	83	69	83.1
	250x002 vs. 002	9 503	6	4	66.7
4	301x005 vs. 301	9 273	99	72	72.7
	301x005 vs. 005	9 467	41	32	78.0
	Total	75 515	433	329	74.8

For the eight hybrid-inbred comparisons sharing the same parent, 0, 15, 21, and 27 genes were differentially expressed in common for inbreds 002, 005, 250, and 301, respectively, the majority for the two dent lines. When comparing the four triplet pairs sharing one parent, 107 genes were differentially expressed in common (average: 26.8 genes / triplet pair), while for those two triplet pairs with no shared parental line, 35 genes were in common (average: 18 genes / triplet pair) (Table 11).

Table 11. Common differentially expressed genes among related and unrelated triplets. TRIP 1: 002_002x301_301; TRIP 2: 005_005x250_250; TRIP 3: 250_250x002_002; TRIP 4: 301_301x005_005; F - flint, D - dent. First inbred denotes the female parent (example: F1D2, F1 flint 1 female, D2 dent 2 male) 002 - flint 1, 005 - flint 2, 250 - dent 1, 301 - dent 2

Triplets	TRIP	Germplasm type (F-flint, D-dent)	Genes in common
Non-related	1-2	F1D2/F2D1	13
	3-4	D1F1/D2F2	22
	average		17.5
Related	1-3	F1D2/D1F1	17
	1-4	F1D2/D2F2	29
	2-3	F2D1/D1F1	32
	2-4	F2D1/D2F2	29
	average		26.8

3.1.2.2 Gene Ontology description

In the eight hybrid-inbred comparisons, 68 genes were significantly differentially expressed in two, 25 genes in three and 6 genes in four comparisons, giving altogether 99 genes. Most genes in two comparisons were found for 301 vs. 002x301 with 301 vs. 301x005 (19 genes), in three comparisons for 005 vs. 005x250 with 250 vs. 250x002 and with 005 vs. 301x005 (5 genes) and in four comparisons for 301 vs. 002x301 with 250 vs. 005x250 with 250 vs. 250x002 and with 301 vs. 301x005 (3 genes) (Annex: Table 8). Gene Ontology assignment (GO) was applied to classify these 99 genes with regard to their molecular function (http://www.tigr.org/tigr-scripts/tgi/GO_browser.pl?species=maize&gi_dir=zmgi). Due to the fact that one gene can have more than one biological function, the number of gene assignments increased to 126. Out of those, 63 genes were GO described, while for another 63 no gene ontology description was found. 38.1 % of the GO described genes were associated with catalytic activity, 33.3 % with binding activity, 12.7 % belonged to the molecular function unknown category, 6.3 % were associated with structural molecule activity, 3.2 % with transporter and translation regulator activity, and 1.6 % with signal transduction and antioxidant activity (Figure 17).

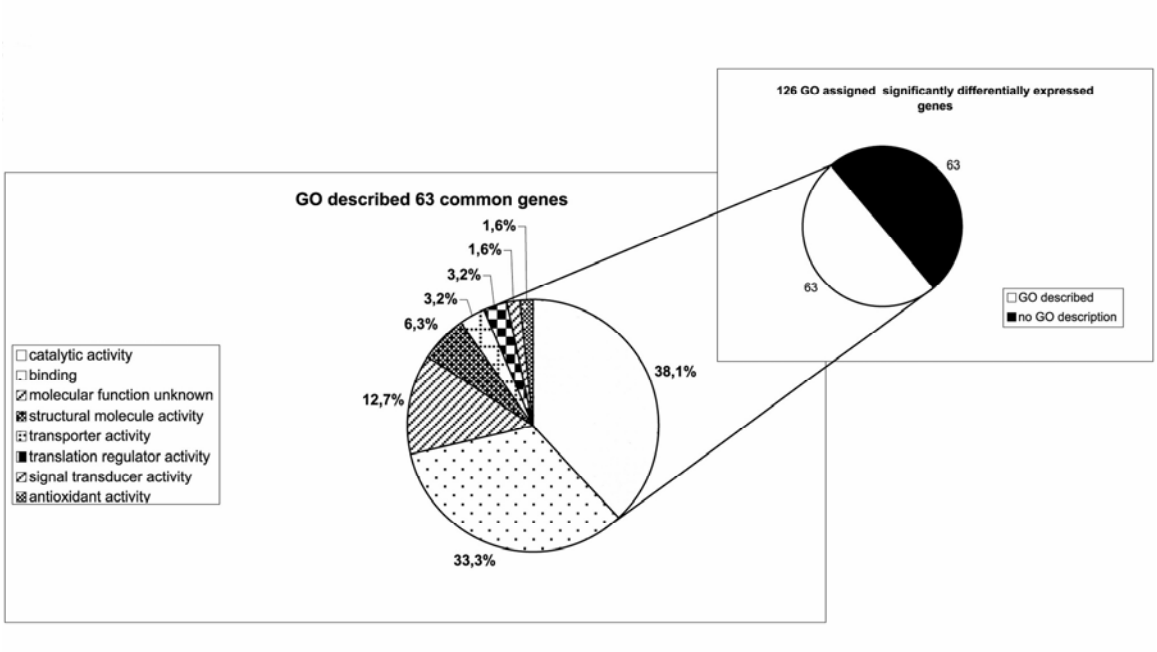

Figure 17. Gene Ontology (GO) description for 63 genes significantly differentially expressed in at least two hybrid - parent line comparisons

Similar distributions, differing slightly in percentages were found for each hybrid-inbred comparison. In order to normalise the obtained GO results to the whole maize gene set with available molecular function assignment (http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/GO_browser.pl?species=Maize&gi_dir=zmgi), the Fisher exact test was performed. Since the p-value obtained was 0.85, the classification into different GO categories was not different from the above mentioned GO classification in maize.

3.1.2.3 Self Organizing Tree Algorithm analysis

Self organizing tree algorithm (SOTA) (MultiExperiment Viewer, TM4 Verison 3.1) analysis was performed on the above mentioned 99 common genes in order to classify them according to their expression patterns. Genes were clustered into 11 sub-clusters containing 46, 2, 4, 7, 10, 15, 1, 2, 4, 4 and 4 genes respectively (Figure 18). Most of the GO described genes (22) were defined in cluster 1 and fell into following categories: 54.5 % catalytic activity, 13.6 % binding and molecular function unknown category each, 9.1 % translation initiation factor activity and 4.5 % transporter and antioxidant activities. Cluster 4 and 7 contained 7 GO described genes each, most of which belonged to catalytic activity, binding and molecular function unknown categories. Cluster 2, 3, 5, 9 and 10 possessed only one to three GO described genes and they belonged in most cases to the above mentioned categories, which confirmed the patterns derived from GO description. Genes in cluster 7, 8 and 11 possessed no gene ontology assignment.

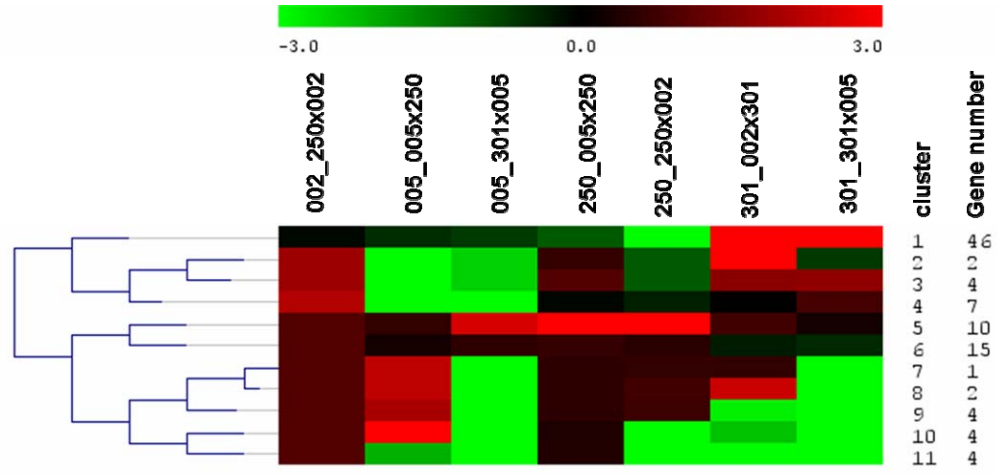

Figure 18. Self organizing tree algorithm (SOTA) applied to 99 genes common between inbred-hybrid comparisons in the heterosis microarray experiment

3.1.2.4 Dominance / Additivity ratios

Out of 37516 differentially expressed gene-triplet combinations (summarized over the four triplets), without significant genotype*environment interactions, 51.2 % genes showed overdominant gene action for mRNA expression levels, with 50.3 % up- and 49.7 % down-regulated genes in the hybrid as compared to the average of both parent lines. 26 % showed a partial dominant expression pattern with 50.2 % genes up- and 49.8 % down-regulated, and 12.6 % had a dominant expression pattern, with 49.6 % up- and 50.4 % down-regulated genes. 10.2 % of all genes displayed additive gene action (Table 12).

Table 12. Dominance/Additivity ratios. D/A ratios were calculated for all genes summarized over the four interpool – triplets, therefore the amount of up/down regulated genes has to be divided by four to calculate the average amount of spots per triplet

Effect	In %	Percentage of up- / down regulated genes	No. of genes	d / a ratio
Additivity	10.2	-	3830	-0.2 - 0.2
Partial dominance	26	50.2 / 49.8	9754	-0.8 - -0.2; 0.2 - 0.8
Dominance	12.6	49.6 / 50.4	4727	-1.2 - -0.8; 0.8 - 1.2
Overdominance	51.2	50.3 / 49.7	19205	< -1.2 - >1.2
Total	100		37516	

3.1.3 Quantitative RT-PCR

In order to accurately quantify expression levels for genes of interest, the endogenous maize actin 1 gene (Mac1) was used for reference. By one-way ANOVA with the confidence level $p < 0.01$ the influence of external factors (in our case various genotypes) on gene expression was tested. The levels of actin in total RNA remained steady ($p = 0.77$), resulting in cycle thresholds (Ct) for qRT experiments of 22.1 to 22.3 for various genotypes (Figure 19). This supports the reliability of the maize actin 1 gene as endogenous qRT-PCR control.

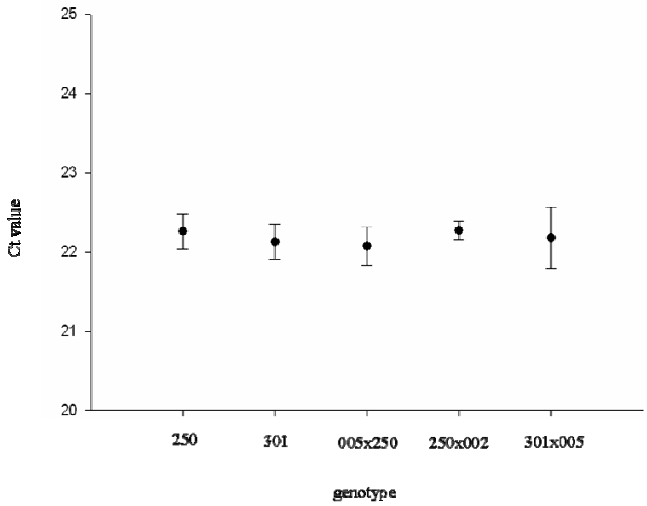

Figure 19. The levels of actin in various genotypes tested in heterosis qRT experiment

Two genes putatively involved in gibberellin biosynthesis and one in jasmonate signalling pathways were chosen for the qRT-PCR experiments. In microarray experiments these genes were differentially expressed in comparisons 005x250 vs. 250, 250x002 vs. 250 and 250 301x005 vs. 301 (Table 13). Differential expression of the gibberellin-stimulated transcript 1 like rice protein homologue was validated by qRT-PCR with a fold change of 10.1 (average from four biological replications) as compared to 2.2 ($p = 0.002$) folds from microarray experiments. The putative plant defensin gene was validated with a fold-change of 2243.1 by qRT (average from four biological replications) as compared to 3.7 ($p = 0.04$) from microarrays. The geranyl-geranyl reductase gene with a fold-change of 3.7 ($p = 0.05$) from microarray experiments was not confirmed to be significantly differentially expressed when averaging fold-changes from all four replications (1.5 by qRT). However, a significant 2.3 fold-change was detected in one of the four replications. In all three cases the up-regulation of genes in a hybrid from microarray experiment was confirmed by qRT.

Coefficients of correlation (R^2) to evaluate the quality of the standard curves for reference and target genes were between 0.98 and 0.99 for all target genes as well for the reference gene, while PCR efficiencies ranged from 1.0 to 1.5 (Table 14, Figures 20 - 22). The gibberellin gene was stably expressed across different greenhouse environments (Denmark, Freising), differing only by 0.9 or 1.0 fold changes between environments for inbreds and hybrids, respectively. The defensin gene varied significantly (20.2 folds) between environments for inbreds but not for hybrids (0.5). The environmental variation for geranyl-geranyl reductase was significant both for inbreds (2.4) and for hybrids (2.5) (Table 14).

Standard curves for genes validated by qRT with corresponding coefficients of correlations (R^2):

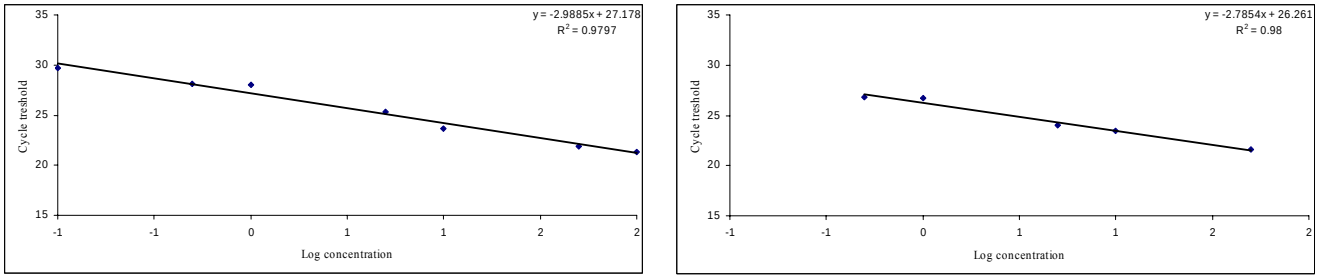

Figure 20. Gibberellin-stimulated transcript 1 like rice protein, target (left) and reference (right) standart curves

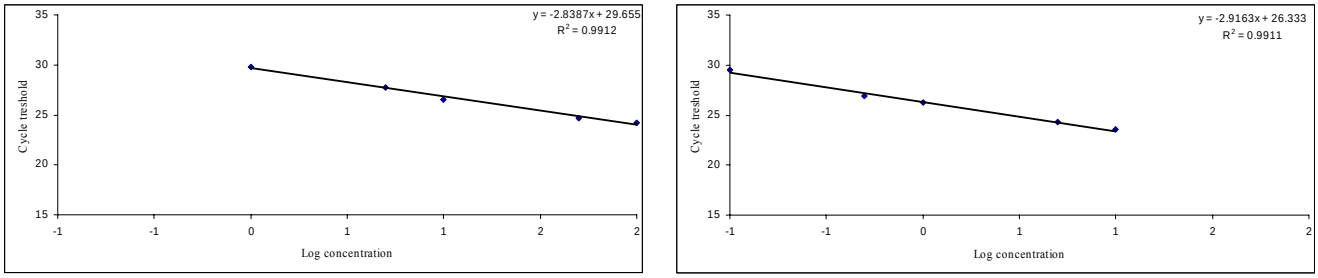

Figure 21. Defensin gene, target (left) and reference (right) standart curves

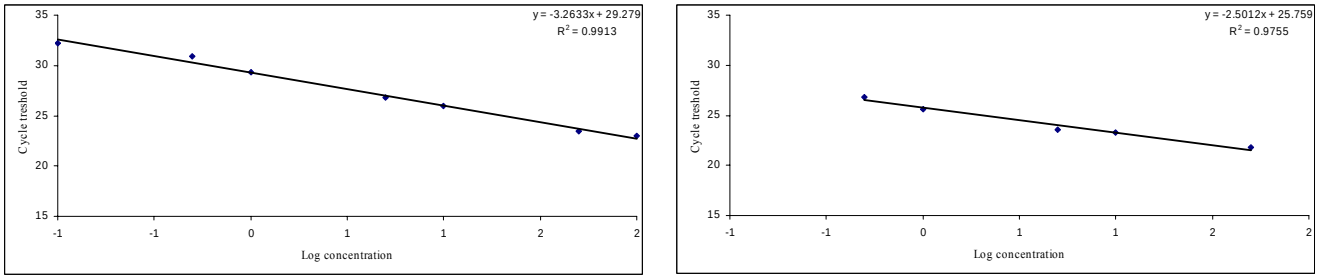

Figure 22. Geranyl-geranyl reductase gene, target (left) and reference (right) standart curves

Table 13. Sequence homologies for differentially expressed genes in relation to PHT heterosis. A limited number of genes were selected from microarray-based expression profiling experiments. Genes and genotypes chosen for qRT-PCR validation based on a fold change, p-value and putative homology are shown in green

Gene EST ID	Genotype	p-value	Microarrays fold of change	GO	TIGR description (homology)
	301x005 vs. 301	0.04205	3.7		
614020G04.y1	250x002 vs. 250	9.07E-05	3.6	Unclassified	putative plant defensin SPI1B {*Picea abies*}, partial (41 %)
	005x250 vs. 250	0.0003	3.3		
MEST59-G12.T3	005x250 vs. 250	0.0006	2	Unclassified	UP|Q8L698 (Q8L698) Defensin precursor, partial (82 %)
	250x002 vs. 250	0.0003	1.9		
496026A12.x1	250x002 vs. 250	0.0500	3.7	Catalytic activity	UP|Q9ZS34 (Q9ZS34) Geranylgeranyl reductase, partial (88 %)
MEST19-G09.T3	002x301 vs. 301	4.93E-08	1.8	Molecular function unknown	UP|O49960 (O49960) Polyphenol oxidase, partial (92 %)
	301x005 vs. 301	6.5071E-10	2		
MEST43-B01.T3	002x301 vs. 301	7.34E-05	2.5	Unclassified	GB|AAA49498.1|213613|QULPROT protamine {*Coturnix japonica*;}, partial (44 %)
	301x005 vs. 301	0.0010	2.1		
MEST283-D07.T3	005x250 vs. 250	0.0032	3.5	Unclassified	GP|5690382|gb|A Pkn10 {*Myxococcus xanthus*), partial (2 %)
	301x005 vs. 301	0.0285	2.8		
707020F12.y1	005x250 vs. 250	1.33E-08	1.9	Unclassified	UP|Q9FYV0 (Q9FYV0) LLS1 protein, partial (14 %) / similar to UP|Q7XC03 (Q7XC03) Putative chlorophyll synthase
605005D02.y2	005x250 vs. 250	0.0015	2.2	Molecular function unknown	PIR|JE0159|JE0159 gibberellin-stimulated transcript 1 like protein - rice {*Oryza sativa*;}, partial (87 %)
614074F08.y3	002x301 vs. 301	0.0004	3.4	Unclassified	(Q8H5X6) Putative NADH dehydrogenase, partial (3 %)

Table 14. Comparison of heterosis microarray and qRT-PCR results

Fold of change / Target EST	Fold of change (microarrays), four biological replications, average	Fold of change (qRT), four biological replications, average	Fold of change (environment) inbreds/hybrids	Coefficients of determination (R) target/reference	PCR efficiencies target/reference
605005D02.y2	2.2	10.1	0.9 / 1.0	0.98 / 0.98	1.2 / 1.3
614020G04.y1	3.7	2243.1	20.2 / 0.5	0.99 / 0.99	1.3 / 1.2
496026A12.x1	3.7	1.5	2.4 / 2.5	0.99 / 0.98	1.0 / 1.5

3.2 SCMV

3.2.1 SCMV phenotype analysis

To evaluate the SCMV infection rate, susceptible F7 $^{SS/SS}$ plants after sample harvesting for expression profiling experiment were grown for additional two weeks to determine mosaic symptoms. 10 out of 32 SCMV infected plants did not show visible systemic symptoms, which was probably due to the fact, that the whole virus was removed while harvesting inoculated leaves.

3.2.2 Expression profiling

3.2.2.1 Hybridization results

The expression profiling study of the virus infection was carried out with five time point experiments (T1 - T5) and mock control (T9). Altogether 144 SCMV arrays were utilised in this experiment (Figure 23).

Figure 23. Hybridized SCMV microarray

3.2.2.1.1 Within time point analysis

For each of the five time points and the mock control, 4578 observations were collected for each pairwise comparison of near-isogenic genotypes within time-points, altogether 27468 observations across all time-points. Out of these, 65 spots showed significant differential expression at the FDR level of $p \leq 0.05$ (Table 15). 52 significantly differentially expressed genes (80 %) were obtained from previous SCMV experiments (Shi C. et al, 2005), 11 genes (16.9 %) were from the RGA collection of CAU, and 2 exons from the eIF3E barley gene showed differential expression. Due to the fact that some genes were expressed in two or more pairwise combinations, in total 28 different significantly differentially expressed genes were identified, which accounted for 3.67 % of 762 spots (excluding controls) printed on the SCMV array.

The majority of significantly differentially expressed genes were found for time point T2 (1 h after infection): 32 genes, which accounted for 0.7 % of 4578 observations, followed by T4 (12 h after infection) with 11 genes (0.24 %), T3 (6 h after infection) with 9 genes (0.2 %), T9 (mock control) with 6 genes (0.13 %), T5 (24 h after infection) with 5 genes (0.1 %), and T1 (before infection) with 2 genes (0.04 %) (Figure 24).

Table 15. 65 significantly differentially expressed sequences within time points identified in the SCMV experiment

EST	Time-point	Genotype 1	Genotype 2	Estimate	StdErr	tValue	Raw p	Fold of change	Upregulated	FDR p ≤ 0.05
605018B03.x1	2	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-1.04731	0.157936	-6.63124	6.75E-11	2.066674	F7 $^{SS/RR}$	2.32E-07
605018B03.x1	2	F7 $^{RR/RR}$	F7 $^{RR/SS}$	-0.96067	0.152924	-6.28206	5.93E-10	1.946219	F7 $^{RR/SS}$	1.66E-06
605018B03.x1	3	F7 $^{SS/SS}$	F7 $^{RR/RR}$	0.836804	0.154433	5.418565	8.32E-08	1.786089	F7 $^{SS/SS}$	0.000152
605018B03.x1	2	F7 $^{SS/SS}$	F7 $^{RR/RR}$	0.816411	0.152924	5.338685	1.27E-07	1.761019	F7 $^{SS/SS}$	0.000206
605018B03.x1	9	F7 $^{SS/SS}$	F7 $^{RR/RR}$	0.714945	0.155292	4.603885	4.94E-06	1.641421	F7 $^{SS/SS}$	0.004848
605018B03.x1	9	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-0.67784	0.152023	-4.45882	9.63E-06	1.599746	F7 $^{SS/RR}$	0.007555
605018B03.x1	3	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-0.59111	0.152023	-3.88833	0.000111	1.506409	F7 $^{SS/RR}$	0.047531
605018B04.x1	2	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-1.3839	0.154755	-8.94255	3.48E-18	2.609735	F7 $^{SS/RR}$	0
605018B04.x1	9	F7 $^{SS/SS}$	F7 $^{RR/RR}$	1.346429	0.155725	8.646212	3.73E-17	2.54282	F7 $^{SS/SS}$	0
605018B04.x1	9	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-1.57141	0.151469	-10.3745	1.58E-23	2.971949	F7 $^{SS/RR}$	0
605018B04.x1	2	F7 $^{SS/SS}$	F7 $^{RR/RR}$	1.277489	0.152317	8.387051	2.82E-16	2.424167	F7 $^{SS/SS}$	2.44E-12
605018B04.x1	3	F7 $^{SS/SS}$	F7 $^{RR/RR}$	1.267925	0.150654	8.416131	2.25E-16	2.40815	F7 $^{SS/SS}$	2.44E-12
605018B04.x1	3	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-1.13147	0.150654	-7.51039	1.84E-13	2.190821	F7 $^{SS/RR}$	8.4E-10
605018B04.x1	9	F7 $^{RR/RR}$	F7 $^{RR/SS}$	-1.03409	0.153094	-6.75459	3.05E-11	2.047821	F7 $^{RR/SS}$	1.2E-07
605018B04.x1	2	F7 $^{RR/RR}$	F7 $^{RR/SS}$	-0.97161	0.154755	-6.27838	6.06E-10	1.961028	F7 $^{RR/SS}$	1.66E-06
605018B04.x1	4	F7 $^{RR/RR}$	F7 $^{SS/SS}$	-0.89492	0.150654	-5.9402	4.52E-09	1.859501	F7 $^{SS/RR}$	1.08E-05
605018B04.x1	5	F7 $^{SS/SS}$	F7 $^{RR/RR}$	0.710469	0.150654	4.715892	2.91E-06	1.636336	F7 $^{SS/SS}$	0.003079
605018B04.x1	3	F7 $^{SS/SS}$	F7 $^{RR/SS}$	0.690527	0.150654	4.583523	5.43E-06	1.613873	F7 $^{SS/SS}$	0.005143
605018B04.x1	5	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-0.65913	0.150654	-4.37512	1.4E-05	1.57913	F7 $^{SS/RR}$	0.010133
606007B06.x1	3	F7 $^{SS/SS}$	F7 $^{RR/RR}$	0.593707	0.103016	5.763245	1.24E-08	1.50912	F7 $^{SS/SS}$	2.62E-05
606007B06.x1	3	F7 $^{SS/SS}$	F7 $^{RR/SS}$	0.419039	0.103016	4.067699	5.29E-05	1.337036	F7 $^{SS/SS}$	0.027941
606021F11.x2	5	F7 $^{SS/SS}$	F7 $^{RR/SS}$	1.220535	0.253576	4.813295	1.86E-06	2.330331	F7 $^{SS/SS}$	0.002316
614013G06.x1	3	F7 $^{SS/SS}$	F7 $^{SS/RR}$	0.713831	0.180833	3.947447	8.71E-05	1.640153	F7 $^{SS/SS}$	0.040567
614044F12.x4	1	F7 $^{SS/SS}$	F7 $^{RR/SS}$	-0.50806	0.094337	-5.38562	9.88E-08	1.422137	F7 $^{RR/SS}$	0.00017
614044F12.x4	1	F7 $^{SS/SS}$	F7 $^{RR/RR}$	-0.4474	0.094337	-4.74261	2.56E-06	1.363582	F7 $^{RR/RR}$	0.002812
614044F12.x4	4	F7 $^{SS/RR}$	F7 $^{RR/SS}$	0.411286	0.094337	4.359771	1.5E-05	1.329871	F7 $^{SS/RR}$	0.010551
614044F12.x4	4	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-0.39051	0.094337	-4.13955	3.91E-05	1.310858	F7 $^{SS/RR}$	0.022349
945031C10.X1	2	F7 $^{RR/RR}$	F7 $^{SS/RR}$	2.926628	0.721324	4.057301	7.15E-05	7.60331	F7 $^{RR/RR}$	0.034466
949062B09.y1	2	F7 $^{RR/RR}$	F7 $^{RR/SS}$	-0.62373	0.145207	-4.29545	2E-05	1.540855	F7 $^{RR/SS}$	0.013406
MEST12-E11.T3	4	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-0.38412	0.073624	-5.21731	2.4E-07	1.305063	F7 $^{SS/RR}$	0.000346
MEST12-E11.T3	4	F7 $^{SS/RR}$	F7 $^{RR/SS}$	0.360367	0.073624	4.89468	1.22E-06	1.283752	F7 $^{SS/RR}$	0.001601

MEST12-E11.T3	4	F7 $^{SS/SS}$	F7 $^{SS/RR}$	-0.35333	0.073624	-4.79917	1.95E-06	1.27751	F7 $^{SS/RR}$	0.002328
MEST12-E11.T3	2	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-0.33297	0.073624	-4.52258	7.18E-06	1.259604	F7 $^{SS/RR}$	0.005974
MEST19-G10.T3	2	F7 $^{SS/SS}$	F7 $^{RR/RR}$	0.337777	0.083843	4.028667	6.23E-05	1.263807	F7 $^{SS/SS}$	0.031991
MEST22-A03.T3	9	F7 $^{SS/RR}$	F7 $^{RR/SS}$	-0.4788	0.115123	-4.15902	3.6E-05	1.393582	F7 $^{RR/SS}$	0.021042
MEST24-E10.T3	2	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-0.40899	0.081293	-5.031	6.22E-07	1.327751	F7 $^{SS/RR}$	0.000854
MEST24-E10.T3	2	F7 $^{SS/SS}$	F7 $^{RR/RR}$	0.325398	0.081293	4.002774	6.93E-05	1.25301	F7 $^{SS/SS}$	0.034013
MEST24-G11.T3	2	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-0.35724	0.07465	-4.78555	2.08E-06	1.280975	F7 $^{SS/RR}$	0.002383
MEST24-G11.T3	2	F7 $^{SS/SS}$	F7 $^{RR/RR}$	0.288913	0.07465	3.870213	0.000119	1.221719	F7 $^{SS/SS}$	0.05026
MEST40-B08.T3	2	F7 $^{SS/SS}$	F7 $^{RR/RR}$	0.425681	0.091814	4.636315	4.23E-06	1.343206	F7 $^{SS/SS}$	0.004307
MEST40-B08.T3	5	F7 $^{RR/RR}$	F7 $^{RR/SS}$	-0.38553	0.091814	-4.19901	3.03E-05	1.306339	F7 $^{RR/SS}$	0.019342
MEST40-B08.T3	2	F7 $^{RR/RR}$	F7 $^{RR/SS}$	-0.36017	0.091814	-3.9228	9.62E-05	1.283577	F7 $^{RR/SS}$	0.042609
MEST40-G05.T3	2	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-0.35593	0.090676	-3.92531	9.52E-05	1.279812	F7 $^{SS/RR}$	0.042609
MEST41-B03.T3	4	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-0.51701	0.128408	-4.02628	6.29E-05	1.430983	F7 $^{SS/RR}$	0.031991
MEST63-E12.T3	2	F7 $^{RR/RR}$	F7 $^{SS/RR}$	0.436653	0.10452	4.177713	3.32E-05	1.353461	F7 $^{RR/RR}$	0.020598
MEST67-A07.T3	4	F7 $^{SS/RR}$	F7 $^{RR/SS}$	-0.42199	0.079319	-5.32015	1.4E-07	1.339772	F7 $^{RR/SS}$	0.000214
MEST67-A07.T3	4	F7 $^{RR/RR}$	F7 $^{RR/SS}$	-0.34131	0.079319	-4.30298	1.93E-05	1.266903	F7 $^{RR/SS}$	0.01323
MEST67-A07.T3	3	F7 $^{RR/RR}$	F7 $^{RR/SS}$	-0.31158	0.079319	-3.92817	9.41E-05	1.241063	F7 $^{RR/SS}$	0.042609
MEST82-F04.T3	2	F7 $^{RR/RR}$	F7 $^{RR/SS}$	0.391448	0.085552	4.575582	5.62E-06	1.311709	F7 $^{RR/RR}$	0.005148
MEST82-F04.T3	2	F7 $^{RR/RR}$	F7 $^{SS/RR}$	0.3907	0.085552	4.566841	5.86E-06	1.31103	F7 $^{RR/RR}$	0.005188
MEST82-F04.T3	2	F7 $^{SS/SS}$	F7 $^{RR/RR}$	-0.34136	0.085552	-3.99016	7.3E-05	1.266954	F7 $^{RR/RR}$	0.034585
MEST333-H11.T3	2	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-0.64441	0.145669	-4.42379	1.13E-05	1.563099	F7 $^{SS/RR}$	0.008593
Zm06_09h07_R	2	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-0.69541	0.117252	-5.9309	4.74E-09	1.619341	F7 $^{SS/RR}$	1.08E-05
Zm06_09h07_R	2	F7 $^{RR/RR}$	F7 $^{RR/SS}$	-0.64707	0.117252	-5.51868	4.82E-08	1.565988	F7 $^{RR/SS}$	9.45E-05
Zm06_09h07_R	4	F7 $^{SS/SS}$	F7 $^{SS/RR}$	-0.48938	0.117252	-4.17378	3.37E-05	1.403843	F7 $^{SS/RR}$	0.020598
PAC000000001182	2	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-0.66483	0.171927	-3.86693	0.000121	1.585384	F7 $^{SS/RR}$	0.05036
946126A02.y1	2	F7 $^{RR/RR}$	F7 $^{RR/SS}$	-0.78649	0.183789	-4.2793	2.15E-05	1.724872	F7 $^{RR/SS}$	0.014038
946126A02.y1	2	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-0.75526	0.18471	-4.08892	4.86E-05	1.687939	F7 $^{SS/RR}$	0.02616
1091032B12.y1 a	4	F7 $^{SS/SS}$	F7 $^{RR/SS}$	-0.8919	0.216297	-4.1235	4.21E-05	1.855617	F7 $^{RR/SS}$	0.023108
1091032B12.y1 b	2	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-0.66695	0.1484	-4.49427	8.24E-06	1.587714	F7 $^{SS/RR}$	0.006655
1091032B12.y1 b	2	F7 $^{RR/RR}$	F7 $^{RR/SS}$	-0.6551	0.1484	-4.41441	1.18E-05	1.574725	F7 $^{RR/SS}$	0.008777
za72g09.b50	2	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-0.52669	0.131339	-4.01017	6.73E-05	1.44062	F7 $^{SS/RR}$	0.033618
946063C12.y1	5	F7 $^{RR/RR}$	F7 $^{RR/SS}$	1.01785	0.261087	3.898514	0.000107	2.024899	F7 $^{RR/RR}$	0.04668
exon 1 (eIF3E barley)	2	F7 $^{RR/RR}$	F7 $^{RR/SS}$	-0.5727	0.126115	-4.54109	6.6E-06	1.487306	F7 $^{RR/SS}$	0.005669
exon 1 (eIF3E barley)	2	F7 $^{RR/RR}$	F7 $^{SS/RR}$	-0.52465	0.126115	-4.16008	3.58E-05	1.438584	F7 $^{SS/RR}$	0.021042

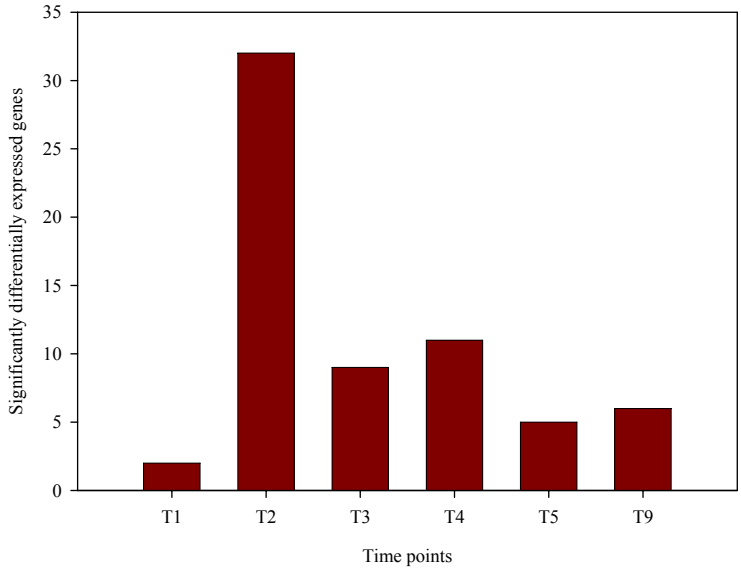

Figure 24. The amount of significantly differentially expressed genes over six time points (including mock control as T9) for the SCMV experiment

Four genotypes (F7 $^{SS/SS}$, F7 $^{RR/RR}$, F7 $^{SS/RR}$, F7 $^{RR/SS}$) give six possible pairing combinations. When considering genotypes combinations most genes were found for F7 $^{RR/RR}$ - F7 $^{SS/RR}$, F7 $^{RR/RR}$ - F7 $^{RR/SS}$, and F7 $^{SS/SS}$ - F7 $^{RR/RR}$, summarising over all time points (Table 16). In pairwise comparisons, 31 genes (corresponding to 13 different genes) were common in two pairwise comparisons, 14 genes (corresponding to 7 different genes) in three pairwise comparisons, and 2 genes in four pairwise comparisons. None of the genes was common between five or all six pairwise comparisons. For pairwise comparisons, most differentially expressed genes were found between genotypes F7 $^{RR/RR}$ - F7 $^{SS/RR}$ with F7 $^{RR/RR}$ - F7 $^{RR/SS}$ (7 genes), for F7 $^{SS/SS}$ - F7 $^{RR/RR}$ with F7 $^{RR/RR}$ - F7 $^{SS/RR}$ (6 genes) and 3 genes for comparisons of genotype F7 $^{SS/SS}$ - F7 $^{RR/RR}$ with F7 $^{SS/SS}$ - F7 $^{RR/SS}$ and F7 $^{SS/SS}$ - F7 $^{RR/RR}$ with F7 $^{RR/RR}$ - F7 $^{RR/SS}$. For three pairings most genes were common for comparisons of genotype F7 $^{SS/SS}$ - F7 $^{RR/RR}$ with F7 $^{RR/RR}$ - F7 $^{SS/RR}$ and with F7 $^{RR/RR}$ - F7 $^{RR/SS}$ (3 genes) and for F7 $^{SS/SS}$ - F7 $^{RR/RR}$ with F7 $^{SS/SS}$ - F7 $^{RR/SS}$ and with F7 $^{RR/RR}$ - F7 $^{SS/RR}$, and F7 $^{SS/SS}$ - F7 $^{RR/RR}$ with F7 $^{RR/RR}$ - F7 $^{SS/RR}$ and with F7 $^{SS/RR}$ - F7 $^{RR/SS}$ (2 genes each). When comparing four gene pairings, F7 $^{SS/SS}$ - F7 $^{RR/RR}$ with F7 $^{SS/SS}$ - F7 $^{RR/SS}$ with F7 $^{RR/RR}$ - F7 $^{SS/RR}$ and with F7 $^{RR/RR}$ - F7 $^{RR/SS}$, and F7 $^{SS/SS}$ - F7 $^{RR/RR}$ with F7 $^{SS/SS}$ - F7 $^{RR/SS}$ with F7 $^{RR/RR}$ - F7 $^{SS/RR}$ and with F7 $^{SS/RR}$ - F7 $^{RR/SS}$, 2 genes were found in common.

Two genes (*605018B04.x1* and *605018B03.x1*) were significantly differentially expressed most commonly within time points and for different genotype pairings, 12 and 7 times respectively (Table 17).

Table 16. The amount of significantly differentially expressed genes for genotype pairs within time points

Time point	Genotype F7 $^{SS/SS}$ - F7 $^{RR/RR}$	Genotype F7 $^{SS/SS}$ - F7 $^{SS/RR}$	Genotype F7 $^{SS/SS}$ - F7 $^{RR/SS}$	Genotype F7 $^{RR/RR}$ - F7 $^{SS/RR}$	Genotype F7 $^{RR/RR}$ - F7 $^{RR/SS}$	Genotype F7 $^{SS/RR}$ - F7 $^{RR/SS}$	Total
1	1	0	1	0	0	0	2
2	7	0	0	16	9	0	32
3	2	1	2	2	1	0	9
4	0	2	1	4	1	4	11
5	1	0	1	1	2	0	5
9	2	0	0	2	1	1	6
Total	13	3	5	25	14	5	65

In the whole set of differentially expressed genes 26.1 % were up-regulated in genotype F7 $^{SS/SS}$, 24.3 % in genotype F7 $^{RR/RR}$, and 24.8 % in genotypes F7 $^{SS/RR}$ and F7 $^{RR/SS}$, each. When considering only significantly differentially expressed genes 24.6 % were up-regulated in genotype F7 $^{SS/SS}$ and F7 $^{RR/SS}$, each, 10.8 % in genotype F7 $^{RR/RR}$ and 40 % in genotype F7 $^{SS/RR}$.

For all differentially expressed genes, 99.1 % showed a fold of change below 2, 0.8 % between 2 and 3 folds, 0.04 % between 3 and 4 folds and 0.002 % over 4 folds. For the 65 significantly differentially expressed genes 83.1 % (54 genes) had a fold change between 1 and 2, 15.4 % (10 genes) between 2 and 3 and 1.5 % (1 gene) between 7 and 8.

Table 17. 28 significantly differentially expressed genes within time-point

EST	Differential expression in time-point	Genotype F7 $^{SS/SS}$ - F7 $^{RR/RR}$	Genotype F7 $^{SS/SS}$ - F7 $^{SS/RR}$	Genotype F7 $^{SS/SS}$ - F7 $^{RR/SS}$	Genotype F7 $^{RR/RR}$ - F7 $^{SS/RR}$	Genotype F7 $^{RR/RR}$ - F7 $^{RR/SS}$	Genotype F7 $^{SS/RR}$ - F7 $^{RR/SS}$
605018B03.x1	2 / 3 / 9	x			x	x	
605018B04.x1	2 / 3 / 4 / 5 / 9	x		x	x	x	
606007B06.x1	3	x		x			
606021F11.x2	5			x			
614013G06.x1	3		x				
614044F12.x4	1 / 4	x		x	x		x
945031C10.X1	2				x		
949062B09.y1	2					x	
MEST12-E11.T3	2 / 4		x		x		x
MEST19-G10.T3	2	x					
MEST22-A03.T3	9						x
MEST24-E10.T3	2	x			x		
MEST24-G11.T3	2	x			x		
MEST40-B08.T3	2 / 5	x				x	
MEST40-G05.T3	2				x		
MEST41-B03.T3	4				x		
MEST63-E12.T3	2				x		
MEST67-A07.T3	3 / 4					x	x
MEST82-F04.T3	2	x			x	x	
MEST333-H11.T3	2				x		
Zm06_09h07_R	2 / 4		x		x	x	
PAC000000001182	2				x		
946126A02.y1	2				x	x	
1091032B12.y1 a	4			x			
1091032B12.y1 b	2				x	x	
za72g09.b50	2				x		
946063C12.y1	5					x	
exon 1 (eIF3E barley gene)	2				x	x	
Total		9	3	5	18	11	4

3.2.2.1.1.1 Gene Ontology description

Maize GO assignment (http://www.maizegdb.org/) was performed on 20 significantly differentially expressed ESTs, for which annotations were available in the maize database. Since more than one biological function might be assigned, the 20 selected genes showed 30 GO hits, within which for 19 GO terms were available and for 11 no gene term (and therefore also no description) was identified. For the 19 described genes, 6 each were assigned to catalytic activity and molecular function unknown, respectively, 4 to transporter activity and 3 to binding activity.

Maize database for functional assignment does not include the category Cell rescue, defence and virulence, therefore genes identified in this experiment were compared with those found by Whitham et al., (2003) for *Arabidopsis*. Thaumatin-like protein, alcohol dehydrogenase, and glutathione S-transferase among other genes with related metabolic pathways were identified as common in both studies and classified into the cell rescue, defence, death, and ageing category.

3.2.2.1.1.2 Map positions

Map positions for 7 out of 20 ESTs were found in the Maize GDB. Seven of the Chinese RGA sequences were mapped (Annex: Tables 9 - 10). For some genes, more than one map position was available. The majority of genes (6) were located on chromosome 8, in a continuous bin 8.04 - 8.06, whereas 3 genes were located on chromosome 10 (bin 10.04) and on chromosome 1 (bin 1.02 and 1.06 - 1.07), respectively. Furthermore, 2 genes were assigned to chromosome 6, carrying the *Scmv1* resistance allele, and another 2 to chromosome 3, carrying the *Scmv2* allele.

3.2.2.1.2 Between time point analysis

14070 genes out of all observations showed significant differential expression between time points at the level of $p \leq 0.05$. When comparing time points, 763 observations of differentially expressed genes for each of the four near isogenic genotypes were obtained. The number of significantly differentially expressed genes within these observations is given in Table 18 and Figure 25.

Table 18. Number of significantly differentially expressed genes within time / time comparisons, out of 763 observations for each genotype

Time-point comparisons	F7 SS/SS	F7 RR/RR	F7 SS/RR	F7 RR/SS	Total
1 - 2	427	427	430	425	1109
1 - 3	496	490	500	489	1975
1 - 4	430	429	416	419	1694
1 - 5	473	479	485	464	1901
1 - 9	418	429	411	416	1674
2 - 3	77	73	87	58	295
2 - 4	142	128	131	138	539
2 - 5	141	118	130	130	519
2 - 9	152	153	138	152	595
3 - 4	125	129	156	116	526
3 - 5	132	142	140	137	551
3 - 9	163	187	195	182	727
4 - 5	58	63	64	53	238
4 - 9	145	157	156	154	612
5 - 9	136	134	116	129	515
Total	3515	3538	3555	3462	14070 / 11780

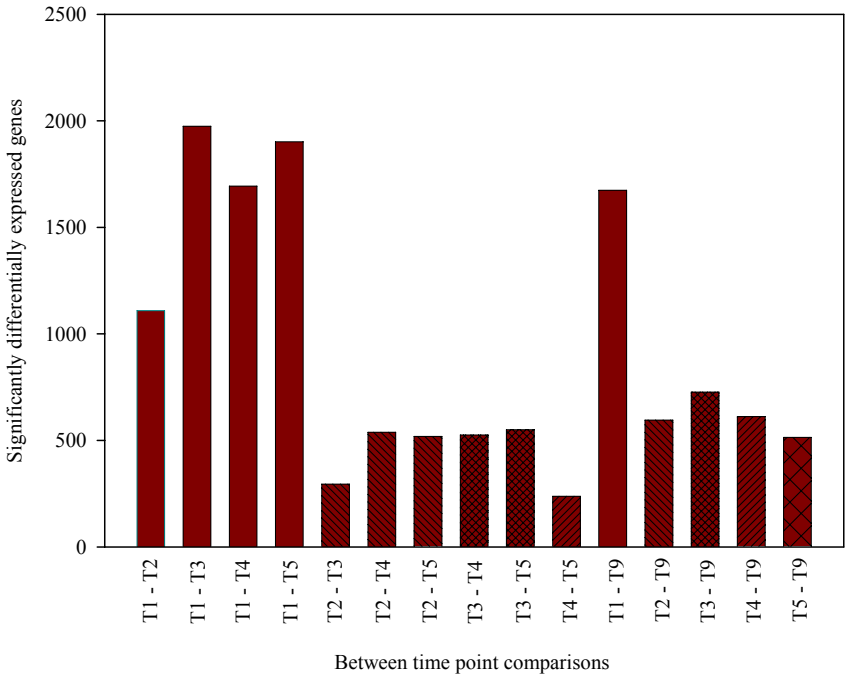

Figure 25. Significantly differentially expressed genes in comparisons between time points across the four genotypes

Out of the total amount of differentially expressed genes 28.9 % were up-regulated in T1, 17 % in T2, 13.0 % in T3, 16.0 % in T4, 12.0 % in T5 and 13.1 % in T9. Among the significantly expressed genes, 62.4 % were up-regulated in T1, 8.2 % in T2, 3.7 % in T3, 9.1 % in T4, 5.7 % in T5, and 11 % in T9.

Across all differentially expressed genes, 73.0 % were expressed below a 2 fold of change, 16.1 % between 2 and 3 folds, 6.2 % between 3 and 4 folds, 2.4 % between 4 and 5 folds and 2.3 % over 5 folds. Across significantly differentially expressed genes, 23.6 % displayed a fold change between 1 and 2, 41.8 % between 2 and 3, 20.1 % between 3 and 4, 7.8 % between 4 and 5, and 7.4 % over 5 fold (Table 19).

Table 19. Distribution of differentially and significantly differentially expressed genes in SCMV experiment regarding folds of change

Genes / Fold change	Differentially expressed genes	F7 SS/SS	F7 RR/RR	F7 SS/RR	F7 RR/SS	%	Significant differentially expressed genes	F7 SS/SS	F7 RR/RR	F7 SS/RR	F7 RR/SS	%
1 - 2	33430	8328	8338	8362	8402	73.0	3223	787	795	831	810	23.6
2 - 3	7364	1822	1846	1854	1842	16.1	5875	1437	1484	1500	1454	41.8
3 - 4	2845	750	709	695	691	6.2	2832	746	707	690	689	20.1
4 - 5	1103	268	308	269	258	2.4	1102	268	308	269	257	7.8
5 and more	1038	277	244	265	252	2.3	1038	277	244	265	252	7.4
Total	45780	11455	11455	11455	11455		14070	3515	3538	3555	3462	

3.2.3 Quantitative RT-PCR

The validation of SCMV resistance related candidate genes was carried out by quantitative RT-PCR on four sequences with homology to genes expressing a metallothionein-like protein, 26S ribosomal RNA, a 14-3-3-like protein GF14-6, and S-adenosylmethionine synthetase 1 (Table 20). These genes were selected for qRT validation based on their map position, expression pattern, fold of change from microarray experiments, and sequence homology to genes involved in resistance response.

Differential expression of the metallothionein-like protein homologue was validated by qRT-PCR with a fold change of 89.2 (average from four biological replications) as compared to 2.6 ($p = 0.0$) folds from microarray experiments. The putative 26S ribosomal RNA gene and the S-adenosylmethionine synthetase 1 gene were not validated when averaging four biological replications (1.6 and 1.2 folds, respectively), but had significant fold of changes in

one of the four replications. In case of 26S ribosomal RNA the significant fold of change was 2.5 in biological replication 1, and for the S-adenosylmethionine synthetase 1 gene the significant fold of change was 2.1 in biological replication 4. The 14-3-3- like protein GF14-6 was not validated by qRT, in any of the four biological replications, but the highest fold value in one replication was 1.7 and 1.6 in another two replications. Coefficients of correlation (R^2) for reference and target genes were between 0.98 and 0.99, confirming good quality of standard curves (Figures 26 - 29), while PCR efficiencies for target and reference genes ranged from 1.0 to 1.4 (Table 21).

Table 20. Sequence homologies for SCMV selected differentially expressed genes in the SCMV experiments

Gene ID	Genotype	Microarrays FDR p-value	Microarrays fold of change	GO	TIGR description (homology)	Map position (bin)
605018B03.x1	$F7^{RR/RR}$ $F7^{SS/RR}$	2.317E-07	2.1	Molecular function unknown	gb|AF036494.1|AF036494 Eucryphia lucida large subunit 26S ribosomal RNA gene, partial sequence, partial (52 %)	1.02 / 6.0 / 7.02
605018B04.x1	$F7^{RR/RR}$ $F7^{SS/RR}$	0	2.6	binding	UP|Q5U7K6_9POAL (Q5U7K6) Metallothionein-like protein, partial (94 %)	-
946126A02.y1	$F7^{RR/RR}$ $F7^{RR/SS}$	0.0140382	1.7	-	UP|METK_ORYSA (P46611) S-adenosylmethionine synthetase 1 (Methionine adenosyltransferase 1) (AdoMet synthetase 1) , complete	8.05
Zm06_09h07_R	$F7^{RR/RR}$ $F7^{SS/RR}$	1.085E-05	1.6	-	UP|14331_MAIZE (P49106) 14-3-3-like protein GF14-6, complete	1.07

Table 21. Comparison of SCMV microarray and qRT-PCR results

Fold of change / Target gene	Fold of change (microarrays), four biological replications, average	Fold of change (qRT), four biological replications, average	Coefficients of determination (R) target/reference	PCR efficiencies target/reference
605018B03.x1	2.1	1.6	0.99 / 0.99	1.1 / 1.2
605018B04.x1	2.6	89.2	0.99 / 0.98	1.1 / 1.4
946126A02.y1	1.7	1.2	0.98 / 0.98	1.0 / 1.1
Zm06_09h07_R	1.6	1.4	0.99 / 0.99	1.1 / 1.1

Standard curves for genes validated by qRT with corresponding coefficients of correlations (R^2):

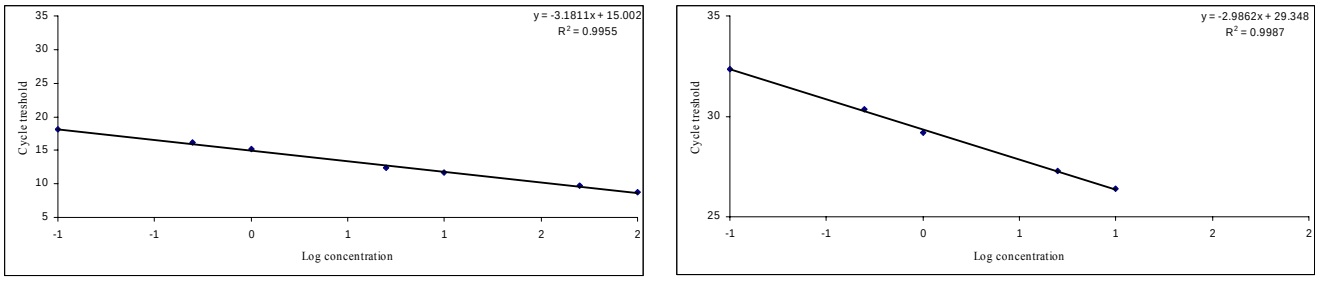

Figure 26. Metallothionein-like protein, target (left) and reference (right) standart curves

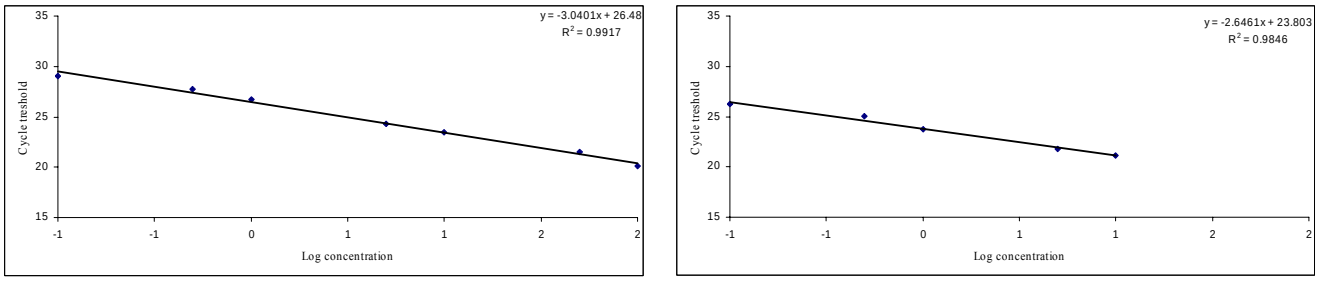

Figure 27. 26S ribosomal RNA gene, target (left) and reference (right) standart curves

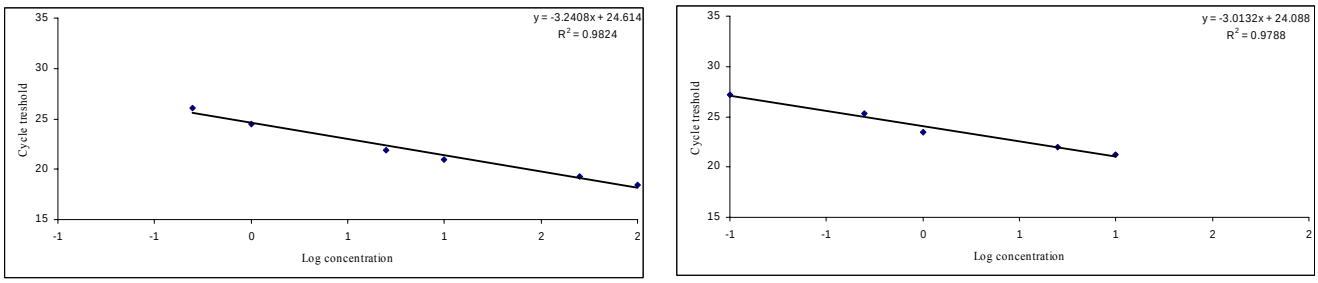

Figure 28. S-adenosylmethionine synthetase 1 gene, target (left) and reference (right) standart curves

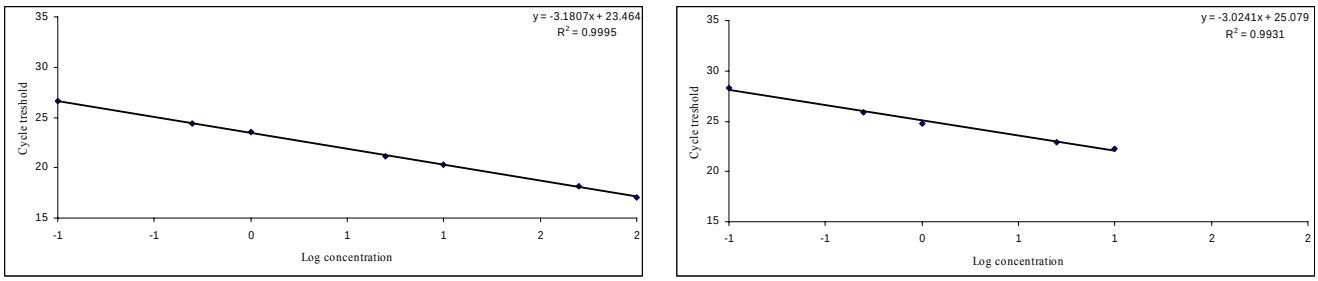

Figure 29. 14-3-3-like protein GF14-6, target (left) and reference (right) standart curve

4 DISCUSSION

4.1 Reliability of expression profiling experiments

Microarrays are widely adopted for the investigation of both vegetative development of the plant, like for instance embryogenesis, grain filling or flowering, and complex interactions dependent on environmental stimuli, for example the influence of plant development by phytohormones or environmental stresses, like salt, osmotic or cold stress (Schnable, 2004).
Because most agronomically important traits are complex inherited, application of global expression profiling seems promising for identification of both expression patterns and candidate genes in organs or tissues of interest.
Reliability of microarray analyses depends on the quality of the experiment, which includes tissue selection, choice of experimental factors, appropriate design including the number of biological and technical replications, quality controls, data monitoring, and data processing (http://www.mged.org/Workgroups/MIAME/miame_checklist.html). Therefore, any micro-array experiment should be further validated by qRT-PCR or northern blotting, to validate differential or non-differential expression of genes identified by expression profiling.

4.1.1 Reliability of microarray experiments

The impact of number of replications and their single or pooled analysis on the final result has been reported by Lee et al., (2000). The authors recommended the usage of at least minimum number of three biological replications, as replications allow a more precise estimation of average values. Moreover, especially for samples from a single specimen, pooling of microarray data from several replications was shown to produce a more accurate picture of gene expression and a reduction in the discovery of false positives and false negatives. In contrary, single microarray output analysis produced substantial gene expression variability. Furthermore, the utilisation of dye-swap replications is strongly advised for any experiment including fluorescent dyes (Kerr et al., 2000; Kerr and Churchill, 2001). Gene expression levels are measured via Cy3 and Cy5 fluorescent dyes. Because of the fact that the two dyes incorporate to the sample with different rates, creating so called “dye biases”, and that the background fluorescence intensity of the Cy3 channel is generally higher than that of the Cy5 channel, the application of dye-swap in an experimental design and its subsequent

normalisation are necessary (Tseng et al., 2001; Yang et al., 2002; Martinez et al., 2003; Raghavachari et al., 2003).

In this study, four biological replications, technical dye-swap replication, triplication of each spot within the SCMV array, as well as utilisation of quality controls were applied in order to monitor and improve the overall precision of the experimental data. Lowess normalisation to correct intensity-dependent ratios (Shi L. M. et al., 2005) and pooled analysis of multiple observations for each spot were employed to maximise the optimality of data analysis. Consequently, the results obtained from the heterosis and SCMV expression profiling assays are supposed to be highly reliable.

4.1.2 Comparison array - qRT

In order to validate differential expression of genes observed by microarray-based expression profiling, quantitative RT-PCR was applied. The method first used by Higuchi et al., (1993), permits with some modifications and advancements measurement of mRNA expression levels, DNA copy number, transgene copy number, expression analyses, allelic discrimination as well as several other applications (Ginzinger, 2002). Moreover, quantitative RT-PCR allows direct measurement of the template amount of the target gene due to its ability to measure the product of a target gene in a linear range of the amplification reaction, which allows reliable data validation (Schmittgen, 2000; Schmittgen and Zakrajsek, 2000). It is the currently the most robust and sensitive technique for validation of findings from expression profiling. Maguire et al., (2002) using cDNA microarrays in combination with qRT in soybean, found a strong linear correlation between microarrays and qRT data, thus confirming the reliability of the combination of both methods. Rajeevan, (2001), and Jenson, (2003) reported that the majority of genes identified by microarray-based experiments can be validated utilising qRT-PCR approach. In their studies up to 80 % of genes were confirmed to be truly differentially expressed. This is in partial agreement with the findings of this study, where 43 % of genes were confirmed in all biological replications and another 43 % in only one. 14 % of genes were non-concordant between both techniques, similarly to 13 - 16 % reported by Dallas et al., (2005) who utilised mammalian oligonucleotide arrays. Different sensitivities of both techniques, as well as the chance that the experiment can be influenced by other factors, such as for instance RNA degradation, contamination or mistaken quantification, must be considered.

4.2 Molecular basis of PHT heterosis in maize

The objective of the phenotypic study was to focus on plant height as "model character" for heterosis. For this purpose plant height and respective component traits such as number, length, and width of internodes between inbred parents and their hybrids were determined.

Owing to the genetic complexity of heterosis formation the choice of appropriate trait, developmental stage and / or plant part for the expression profiling analyses is arguable.

A trait, to be considered valuable for complex inherited traits analyses must be characterized by sufficient heritability levels. Heritability estimates the relative contributions of differences in genetic and non-genetic factors to the total phenotypic variance in a population. In this study, heritabilities were high and ranged from ~84 to 97 % for PHT, 94 to 99 % for NOI and 71 to 97 % for INT3 - 5, 8 and 9, but were lower for INT1, 2, 6, and 7. Therefore, INT1, 2, 6, and 7 were not analysed in detail. Similar, high heritability values for PHT were also observed by Betrán, (2003) and Hallauer, (1999) in maize, and by Oettler, (2002) and Herrmann, (2007) in triticale.

Hybrids had generally more and longer internodes as compared to inbred parents. Moreover, significantly higher hybrid PHT in field vs. greenhouse conditions observed in this study led to a substantially increased MPH and HPH for PHT in the field. Increased hybrid PHT heterosis in the field was likely due to a higher sensitivity of inbreds as compared to hybrids against abiotic stress, such as low spring temperatures in the field. Low temperatures present at early stages of plant growth could delay stretching of internodes in sensitive genotypes in this period, thus reducing the overall plant height, as reported by Sari-Gorla et al., (1999) and Sowiński et al., (2005). The sensitivity of inbred lines observed in this study resulted in comparatively large differences in MPH for INT8 and INT9 in the field as compared to the greenhouse, as INT8 and INT9 were the lowermost and earliest elongating internodes. Reduction of inbred's PHT was a consequence of either reduction in length of particular internodes or change in internodes number. Poor performance of tropical maize inbreds under severe stresses and environmental conditions, and dwarfism caused by environmental stress, have also been observed (Zsubori et al., 2002; Betrán et al., 2003; Tollenaar et al., 2002). Heterosis is thought to be more pronounced under stress conditions within the limits regarded to plant physiology and environment (Troyer, 2006).

In conclusion, PHT heterosis seems to be due to two major components: (1) stress dependent heterosis acting through a comparatively high sensitivity of inbreds against stress, and (2) stress-independent heterosis also present under "optimal" growing conditions.

Significant correlations of PHT with lowermost internodes (INT8 and INT9) were detected in both field and greenhouse experiments. However, no specific internode across four triplets was found to explain most of the PHT heterosis in both environments. Since lowermost internodes are formed early in plant development at the terminal spikelet stage of the apical meristem, this tissue was chosen for further investigations in expression profiling experiments. We assumed that at this stage of meristem development, molecular processes affecting plant height might have already been initiated. Furthermore, maize meristems older than around three weeks start turning into floral organ formation (data not shown). In *Arabidopsis,* heterosis for rosette diameter was determined nineteen days after sowing and size differences were established as early as during seedling development (Meyer et al., 2004). Because the aim of the study was to focus on the non-stress related component of heterosis the controlled greenhouse conditions for expression profiling analyses were chosen.

Large sequencing projects on prokaryotic and eukaryotic genomes introduce sizable amounts of new genes. Interpretation of the genome sequence requires, however, creation of a link between sequence and gene function. Identification of the role of newly discovered genes can be done for instance by expression analyses, cellular localisation or biochemical pathways investigation (Renesik and Buell, 2005). Once the sequences of interest are available, they can be spotted onto a cDNA array and their expression profiles can be analysed to dissect gene function (Zinselmeier et al., 2002; Renesik and Buell, 2005). Microarrays provide information about steady-state mRNA abundance in a particular developmental stage, under stress or as a consequence of particular environmental signal and deliver the information about multiple genes simultaneously (Tretheney, 2001).

In this study, transcript abundances between inbred lines and their hybrids were analysed in order to identify candidate genes related to plant height heterosis, to investigate expression patterns and to relate expression profiles to heterosis hypotheses.

433 genes were detected as significantly differentially expressed between in total four inter-pool hybrids vs. their parental inbred comparisons, with 99 genes common between at least two comparisons. Almost 75 % of the 433 genes were up-regulated in a hybrid, which might explain the increased vigour of the hybrids. The superiority of the hybrid over the parental inbreds on the mRNA quantity level was previously reported (Romagnoli et al., 1990; Leonardi et al., 1991; Tsaftaris et al., 1995). The assumption that the two parental alleles in the hybrid may be regulated at the cumulated transcript level and create a combined allelic expression pattern was assumed to be associated with heterosis (Guo et al., 2004). On the

other hand, however, the changes in transcript abundance must not necessarily correlate to the function within the questioned biological process, as reported by Guo et al., (2006). This could happen because of differential expression of genes employed in tissue- or cell-specific expression patterns or due to the fact that not only transcriptional controls dictate the phenotypic value (Trethewey, 2001).

Differentially expressed genes were annotated according to Maize Gene Ontology assignment with regard to their molecular function. Complete or partial annotations for agronomical important crops are available in online databases. Global assignments of genes into categories, such as molecular function, biological process or cellular component are, therefore, feasible (http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/GO_browser.pl?-species=Maize&gi_dir=zmgi). The largest group of genes identified in this experiment (38.1 %) contained sequences classified into the catalytic activity category. Catalysis is a biological process decomposing macromolecules into smaller units to provide chemical energy as required for maintenance of living cells. Up-regulation of genes assigned to this category in hybrids may thus lead to faster growth and development as compared to the parental inbreds. The second largest group included genes with binding activities (33.3 %). Specific binding of vast majority of molecules, like for instance nucleic acids, nucleotides, lipids, proteins or metal ions is necessary for carrying out metabolic processes in the plant. The third analysed group contained genes with unknown molecular function (12.7 %). Around 30 % of all sequences available for *Zea mays* correspond to this group. For some genes classified into this category, promising sequences were found based on homology that might affect hybrid vigour, for example a gene with similarity to a gibberellin-stimulated transcript 1 like protein. Homologous sequences from chromosomal DNA or ESTs existing in fully sequenced model organisms like rice or *Arabidopsis* can be utilised to predict the function of unknown genes (Kohane, 2003; Feng and Mundy, 2006). However, for several genes homology to any functionally described genes is lacking (Feng and Mundy, 2006). Moreover, even if available, homology information can not always be utilised. For example, *Arabidopsis* genes for flowering time (*FRI* and *FLC*) were not at all found in monocotyledonous species or a major day light response gene in barley was not identified to play major role in *Arabidopsis* and rice.

In addition, higher numbers of differentially expressed genes in common in related (same parental contributor) as compared to unrelated triplets were identified in heterosis investigations. None of the genes was differentially expressed in all triplets. Thus, no obvious key genes controlling PHT heterosis in maize emerged from presented study. It is, therefore,

concluded that each triplet possesses its own specific expression pattern, making the prediction of high-performing inbred parent combinations by expression profiling unlikely. However, when utilising linear regression models, Stokes et al., (2006) demonstrated that the prediction of hybrid performance based upon the transcriptome characteristics of inbred lines in *Arabidopsis* was possible. Moreover, the authors applied the method to identify genes showing correlation of transcript abundance in inbred lines with the magnitude of heterosis in maize, thus making the qualitative prediction of yield in maize hybrids feasible.

Furthermore, the question whether heterosis is a general phenomenon or whether different heteroses at molecular level are possible, is arguable. Studies conducted on selected human and animal genes reviewed by Comings and MacMurray, (2002) revealed up to 50 % sequences exhibiting heterosis at molecular level. Moreover, molecular heterosis was presumed to be gene-, phenotype-, gender- and organ specific. No study concerning this subject in plants has yet been released. If this appears to be common, the prediction of model traits and model organisms for heterosis prediction would be unlikely, as for every type of heterosis the constellation would have to be studied "*de novo*".

Heterosis is a greatly complex process, hence the identification of sets of genes investigated under questioned conditions and on a limited number of genotypes, can be assumed to be only a "tip of an iceberg" in the attempt to understand the molecular basis of this phenomenon.

4.2.1 Heterosis hypotheses

The molecular basis of heterosis is of pivotal interest for plant breeders in terms of long-term breeding strategies. Three main models, dominance (partial-dominance), overdominance, and epistasis hypothesis are being discussed as explanation for hybrid vigour in plants. If heterosis is due to dominance, high-performing inbreds exceeding hybrid values are theoretically possible, in contrast to overdominance (Tsaftaris, 1995).
The ratio of dominance to additive effects is being calculated to assign genes in regard to their mode of inheritance. The analysis of d / a ratios was performed on the whole set of differentially expressed genes over the four interpool – triplets in order to give a general impression on how did the hybrids perform as compared to their parental inbred lines. Out of 37516 genes differentially expressed between inter-pool hybrids vs. parental inbreds, more than half exhibited overdominant gene action, 26 % partial dominant, 12.6 % dominant and 10.2 % additive effects with regard to heterosis. Within each group, the number of genes up-

and down-regulated in hybrids was about 50 %. Auger et al., (2005), based on gel blot analyses concluded that non-additive gene expression in diploid and triploid hybrids of maize play the predominant role. In contrary, Swanson-Wagner et al., (2006) in their microarray study utilising the same genotypes, observed only a limited percentage of genes inherited non-additively, within which over- and underdominance played the minor role as compared to dominance. The inconsistency of both studies might have arisen due to differential sampling, diverse environments for plant growth, and / or plant harvesting at different developmental stages. Results similar to Auger et al., (2005), suggesting overdominance as major factor for heterosis for PHT and internode length in maize were presented by Stuber et al., (1992), based on QTL analyses utilising single-marker method and interval mapping. Furthermore, Xiao et al., (1995) conducting a quantitative study on rice claimed dominance to be the main effect. Additionally, Yu et al., (1997), Li et al., (2001) and Luo et al., (2001) suggested epistatis and epistasis in combination with overdominance, respectively to play a main role in generation of heterosis for grain yield components in rice. As a conclusion, the work of Troyer, (2006) reported that multiple types of gene action, such as dominance, additive and epistatic effects might increase yield of hybrid corn.

The findings from the presented heterosis expression profiling experiments are in better agreement with the overdominance as compared to the dominance hypothesis. However, it is unclear yet, how the mode of gene action at the transcript level translates into heterosis for morphological characters.

4.2.2 QRT-PCR validation of microarray data

Sequences with relationship to genes affecting plant height, with interesting expression profiles and with high, statistically significant fold changes ($p \leq 0.05$) were selected for validation. It was assumed that sequences with at least two-folds of change obtained from expression profiling analyses are likely to be validated by quantitative RT-PCR and that the validation of such results it highly reliable.

At first, the choice of an appropriate endogenous control for qRT was essential. Actins are among the most commonly used and reliable references for quantification experiments, therefore the maize actin gene was chosen as an endogenous control (Thellin et al., 1999; Schmittgen and Zakrajsek, 2000; Kim et al., 2003; Brunner et al., 2004; Abruzzo et al., 2005). The stability of actin was confirmed in this experiment with distinct genotypes and within various environments.

For the validation of heterosis expression profiling results genes coding for the gibberellin-stimulated transcript 1-like rice protein (GASR1) and a geranylgeranyl reductase (CHL P) were selected, as both belong to the pathway for gibberellin (diterpens) biosynthesis (Jacobs, 1997). Gibberellins regulate plant development and growth and were found in higher concentrations in hybrids as compared to either parental inbred, which might involve them in regulation of hybrid vigour in maize (Rood et al., 1983 a, b). The GASR1 gene was confirmed to be 10-times higher expressed in a hybrid by qRT (pool of four biological replications from two environments, triplet 005x250 vs. 250) as compared to two-fold in microarray experiments and was both environmentally stable and repeatable. Geranylgeranyl reductase catalyses the reduction of geranylgeranyl diphosphate to phytyl diphosphate, which takes part in porphyrin and chlorophyll metabolism. Reduced activity of geranylgeranyl reductase causes loss of chlorophyll and tocopherols required in photosynthetic reactions (Tanaka et al., 1999). The geranylgeranyl reductase gene with a fold of change 1.5 from microarrays was not confirmed to be differentially expressed as average from four biological replications. However, a significant fold-change of 2.3 was detected in one replication. The third selected gene was a putative defensin. Defensins are involved in the jasmonic acid signalling pathway and jasmonates, “non-traditional plant hormones” are key regulators in developmental, physiological, defence and signalling network processes (Creelman and Mullet, 1997; Lincoln and Zeiger, 1998). The expression of the putative defensin gene showed a fold change of 2243 by RT-PCR as compared to 3.7 in microarray-based expression profiling experiments. The absolute expression of parental inbred was close to zero. Significantly greater fold change results obtained from qRT-PCR as compared to microarray experiments have previously been reported (Yuen et al., 2002; Dallas et al., 2005). Both defensin and geranylgeranyl reductase genes showed differences between environments (Freising and Denmark), either for hybrids, inbreds or for both, which might result from different light, temperature and other regimes at both locations. The up-regulation of the three genes in a hybrid found by qRT was in agreement with microarray experiments.

The very high fold-change differences between both techniques for the heterosis related defensin gene might be explained by 1) the close to zero expression of the defensin gene in inbreds resulting in large variation for ratios for expression levels between hybrids and inbreds, 2) limited sensitivity of microarray technique to detect high differences between samples, 3) any kind of not observed infection of plants in the greenhouse, 4) putative simultaneous detection of different gene family members by microarrays, and 5) incomplete primer specificity, since primers were designed based on public unigene sequences but not

sequences from the genotypes studied. The first reason seems to be most likely, causing high variability in results from both techniques, although we cannot rule out the other explanations. Moreover, the importance of verification of genes belonging to multigene families was pointed out by Maguire et al., (2004). Generally, the range of expression in qRT-PCR analyses is much greater than in the corresponding microarray experiments and the variability might be due to the difference in sensitivity of both techniques, but also to cross-hybridizations with other gene family members for the microarray technique (Rajeevan et al., 2001; Jenson et al., 2003; Chen et al., 2005).

The lack of validation of geranylgeranyl reductase gene could be explained by the fact that 1) the probability of finding differential expression for genes at the significance threshold ($p = 0.05$) is most difficult due to a comparatively high risk of the type I statistical error, or 2) the finding of differential expression for CHL P was a false positive result in microarray experiments or 3) that the pooled analyses of biological replications from microarray experiments could not reveal significance in particular replications.

Based on the results presented above it is presumed, that the usefulness and reliability of the combination of the two expression profiling methods for the successful selection of candidate genes involved in questioned processes is sufficient and reliable.

4.3 Molecular basis of potyvirus resistance

4.3.1 SCMV time course experiment

SCMV expression profiling experiment was carried out on four near isogenic lines, carrying SCMV resistance alleles fixed for *Scmv1* and *Scmv2* regions identified in previous QTL analyses (Melchinger et al., 1998; Xia et al., 1999; Yuan et al., 2003). 65 sequences were identified to be significantly differentially expressed in the SCMV within time point experiment and were annotated according to Maize Gene Ontology Assignment. The majority of up-regulated genes were found for genotype F7 $^{SS/RR}$, carrying *Scmv1* resistance allele on chromosome 6, thus suggesting a probable stronger influence of this locus in creating resistance responses.

The major groups of genes were classified into catalytic activity, similarly to heterosis studies, and molecular function unknown (both 31.6 %), whereas the third group included genes encoding transporter activity (21 %). Based on the fact, that the maize database for functional assignment does not include the cell rescue, defence and virulence category, which is of

special interest for pathogenesis related studies, genes identified in this experiment were additionally compared with those found by Whitham et al., (2003) in *Arabidopsis*, when infecting plants with five distinct viruses, including one mosaic potyvirus. Three identical genes were found and classified into the cell rescue, defence, death, and ageing category from *Arabidopsis*, thus indicating the reliability of the microarray technique for detecting genes related to pathogenesis.

Systemic infection is the ability of a virus to move within the host from primary infection sites to distal regions. The multiplication of the virus and its further spread is a complicated process including many specific gene products. The virus spreads first locally from cell to cell through plasmodesmata, and further over longer distances through phloem (Carrington et al., 1996; Cruz, 1999). In several observations (Lei and Agrios, 1986; Law et al., 1989; Kovács et al., 1998; Kuntze et al., 1997; Grumet et al., 2000) the long-distance movement of the virus in lines resistant to MDMV and SCMV into newly developing parts was inhibited, whereas replication in infected leaves of resistant genotypes was possible. The time course and cell types inhibiting SCMV spread in the inoculated leaves has so far not been determined (Pokorny and Porubova, 2006). The objective of the SCMV experiment was to identify differentially expressed genes and gene cascades over time, in order to uncover genes and signalling pathways involved in host-plant response to viral infection.

The majority of significantly differentially expressed genes within time points were identified 1 h after infection, dropping down to about one-third at 12 hours after infection. Similar results were obtained from between time point experiments, where the majority of induced genes were identified between “before infection” with 1 or 12 hours after infection. This indicates a rapid and dynamic reaction of the plant against the invader as a key step in fast activation of defence mechanisms. It has been reported, that host-pathogen contact changes dramatically the host gene expression while activating immediate production of reactive oxygen intermediates, which switch on defence mechanisms and secondary metabolites synthesis. At the same time, housekeeping genes are being activated to face the demand for energy and biosynthetic pathways to fight off the pathogen attack, followed by the activation of specific genes coding for defence and rescue responses (Scheideler et al., 2002; de Torres et al., 2003; Whitham et al., 2003). In the study of de Torres et al, (2003), the changes in gene expression of *Arabidopsis* upon bacterial infection were detected as fast as within 2 hours after inoculation. In contrary, Scheideler et al., (2002) observed only small percentage of transcripts being induced within 2 hours after infection with *Pseudomonas syringae*, but significantly increasing between 2 and 7 hours, to drop back between 7 and 24

hours after infection. This indicates that the time required for the initiation of defence mechanisms is species, pathogen or species-pathogen dependent.

Furthermore, one-fourth of genes identified in this study showed increased expression at multiple time points (at least two). It is, therefore, assumed that the host gene expression over time points is a complex reaction, which is in agreement with the findings of Whitman et al., (2003) on *Arabidopsis* infected with five various positive-stranded RNA viruses.

Genetic map positions for 14 out of 28 sequences identified to be significantly differentially expressed in the SCMV within time point expression profiling experiment were obtained. Surprisingly, the majority of identified genes were located outside the introgressed *Scmv1* and *Scmv2* regions. A continuous bin 8.04 - 8.06 (chromosome 8) included 6 genes, whereas bin 10.04 (chromosome 10) and bins 1.02, 1.06 - 1.07 (chromosome 1) 3 genes, respectively. Only 2 genes were assigned to chromosome 6, carrying the *Scmv1* resistance allele, and another 2 to chromosome 3, carrying the *Scmv2* allele. One possible reason is the utilisation of near isogenic lines (NILs), which might contain additional donor segments outside the target regions *Scmv1* and *Scmv2*. To produce NILs, a backcross procedure is used to introgress a small region of a donor genome containing a specific allele into an acceptor genome with a different allele (Tuinstra et al., 1997). With every backcross, the amount of donor DNA surrounding the allele of interest but also on other chromosomes is reduced. The retained regions may contain: a) any genes introduced by chance, or b) modifier genes required for the expression of the resistance phenotype. An example for such interaction is the *Mla* gene responsible for resistance to powdery mildew fungus in barley. To provide resistance, *Mla* requires the function of other genetic elements, *Rar1* and *Rar2* (Torp and Jorgensen, 1986). Identification of 6 genes in a tight bin on chromosome 8, and another 3 genes in the same bin on chromosome 10 seem, however to be more in agreement with the modifier gene hypothesis. To prove it, marker screening of the donor line, for the presence of the same segments on chromosomes 8 and 10 should be performed.

4.3.2 QRT-PCR

The validation of differentially expressed genes within time points after infection with SCMV was of special interest, as genes localised outside the *Scmv1* and *Scmv2* regions could putatively contribute to SCMV resistance. Only genes significantly differentially expressed within time points for two near isogenic lines, preferentially for comparisons of F7 $^{RR/RR}$ with F7 $^{SS/RR}$ or F7 $^{RR/SS}$ were considered for validation. Selection of genes for qRT-PCR validation was based mainly on their map position and interesting expression pattern. Four out of the 65 microarray consistently differentially expressed sequences were selected for qRT validation. The 26S ribosomal RNA gene was chosen because of its map position localised on chromosomes 1 and 6 and high fold change. A sequence putatively expressing a metallothionein-like protein was selected due to its high fold change and expression at all time points between four out of six genotype combinations. A sequence putatively expressing a 14-3-3-like protein GF14-6 was selected due to its location on chromosome 1 and 10 and S-adenosylmethionine synthetase 1 due to its expression pattern. A major resistance QTL (*Scmv1)* have been previously mapped and confirmed to be positioned on chromosome 6 (Xia et al., 1999; Xu et al., 1999; Dussle et al., 2000; Zhang et al., 2003), while three minor QTL were mapped on chromosomes 1, 5 and 10 (Xia et al., 1999).

The gene coding for metallothionein-like protein was confirmed to be significantly differentially expressed between F7 $^{RR/RR}$ and F7 $^{SS/RR}$ with a fold of change of 89.2 averaged from four biological replications as compared to 2.6 from microarrays. Only in one of the four biological replications differential expression for the 26S ribosomal RNA and the S-adenosylmethionine synthetase 1 genes were confirmed. The 14-3-3-like protein GF14-6 did not reach the significance level of 2.0 folds of change in neither replication. In all three latter cases, fold of changes were low in the microarray experiments (despite of significance), which might be difficult to reproduce by other methods if close to significance threshold. Similar findings were reported by Czechowski et al., (2004) and Dallas et al., (2005), indicating that genes with higher expression levels from microarray experiments (> 1.5 folds of change) are more likely to be validated by qRT-PCR. However, it cannot be excluded that significant differential expression for at least one of these genes was false positive.

4.3.3 SCMV expression data validation by MDMV experiment

Additional support to the differentially expressed genes identified in the SCMV experiment was delivered by the expression profiling analysis of MDMV virus infection.
The experiment was carried out with two near isogenic genotypes (fully susceptible F7 $^{SS/SS}$ and fully SCMV resistant F7 $^{RR/RR}$), three time points and mock control. 24 SCMV arrays were utilised for hybridization. Only three spots, corresponding to two genes, were identified to be expressed at a significant FDR level of $p \leq 0.05$, two of which in comparison to the mock control and one (metallothionein-like protein) 6 hours after virus infection. All three sequences were up-regulated in the susceptible F7 $^{SS/SS}$, with maximum fold change of 2.5 (data not shown).
The MDMV experiment was set up to compare response of isogenic lines to related but different viruses, assuming that F7 $^{RR/RR}$, containing the *Scmv1* and *Scmv2* regions from the resistant FAP1360A inbred was fully resistant to both SCMV and MDMV, as indicated by Xing et al., (2006). However, recently it was found that F7 $^{RR/RR}$ is susceptible against a new MDMV isolate, used in this experiment (personal correspondence with SCMV lines developers, Research Centre Flakkebjerg, Denmark). It is well known, that plants and pathogens lead an endless survival battle in the nature. It might have happened that a new virulence gene could have evolved in the MDMV isolate, in order to break plant resistance. It was previously speculated that, the more aggressive the pathogen is, the more major genes a host-plant needs in order to be resistant (Bartual et al., 1991).
As F7 $^{RR/RR}$ was unintentionally susceptible to the new MDMV isolate, the findings of only very few differentially expressed genes are in agreement with the lack of difference between the two isogenic lines for MDMV resistance in contrast to the SCMV experiment, and supports the reliability of SCMV results.

4.4 Applications of expression profiling in plant breeding

Microarrays are presumed to be an appropriate tool for the identification of candidate genes for different traits of interest. Whereas the majority of today's applications of microarray analyses is utilised in mammalian research (around 90 %), great progress has been made to adopt the technology in plant studies in recent years (Schena, 2003).
Once candidate genes from microarray studies have been identified, they must be further validated by other methods, such as qRT-PCR or northern-blotting. The success at this step

does not, however, ensure that a gene regulating a particular trait was discovered. To assign the role of a candidate gene to a biological function in complex organisms, subsequent investigations "*in vivo*", like transformations or mutant analyses are crucial (Zinselmeier et al., 2000; Roda and Baldwin, 2003; Hoffmann et al., 2006; Baulcombe, 2004).

Products of single genes are usually easily transferred to other plant species (McManus et al., 1994; Mochizuki et al., 1999). Most common transformation methods include gene transfer between organisms utilising Ti plasmid DNA (first reported by Davey et al., 1980; Krens et al., 1982), cells transformations by *Agrobacterium* T-DNA (Rhodes et al., 1988; Hooykaas, 1989), biolistic or microprojectiles (Klein et al, 1987). Successful reports on the usage of these methods have been reported. Transgenic maize germplasm was enhanced in number of agronomic traits, like insect and herbicide resistances (Bt maize), fungal and bacterial resistances, male sterility for hybrid seed production, metabolic properties of zein proteins and for the lysine and tryptophan content, leading to the development of quality protein maize (QPM) (Gepts, 2002; Paliwal, 2000 c; Geraghty et al., 1981; Pedersen et al., 1982). The limitation of transformation method is, however, the possibility of manipulating only few genes. T-DNA insertions directly into the gene of interest, which can disrupt gene function are effective in case of fully sequenced genomes of *Arabidopsis* or rice.

A RNA knock-out, also called RNA interference (RNAi) is a post-transcriptional gene silencing method used for the analysis of gene function in various organisms (Kusaba, 2004). It is based on an inactivation of the endogenous gene by the anti-sense copy of the same gene leading to its inhibition by self-complimentary RNAs (Waterhouse et al., 1998; Wesley et al., 2001). The phenomenon was first discovered in warms (*Caenorhabditis elegans*) and nowadays is well-known in both, animal and plant kingdom. RNAi can confirm the importance of a given signalling pathway and uncover other components that may play a role in this pathway (Roda and Baldwin, 2003). The method was broadly applied in many organisms, for instance in *Arabidopsis* to investigate the potential of double-stranded RNAi with gene activity (Chuang and Meyerowizt, 2000), in rice to investigate the mechanisms for gene silencing for the glutelin multigene family (Kusaba et al., 2003), or in maize to uncover the function of genes discovered by expression profiling platforms (Cigan et al., 2005). An interesting example of the usage of the method for gaining strain-specific resistance of tobacco to tobacco etch virus (TEV) was delivered by Lindbo et al., (1993) and Mueller et al., (1995). Transgenic plants, carrying a full-length form of the TEV virus coat protein and displaying typical virus symptoms gradually recovered the infection and produced virus free newly developed plant parts. The tobacco study is a type of the anti-viral function of RNA

silencing due to co-suppression. Co-suppression occurs when multiple copies of a gene, homologous to the endogenous gene are inserted in order to enhance its expression. To avoid the co-suppression effect, it is recommended to implement a copy slightly differing in the sequence, most likely from other species (Roda and Baldwin, 2003).

The utilisation of the two described transgenic methods could be applied to reveal the contribution of the gibberellin-stimulated transcript 1-like rice protein (GASR1) in plant height heterosis creation in maize. Furthermore, the 26S ribosomal RNA gene could be utilised to approve or disapprove the antiviral function of this gene in ribosome depurination and blocking the translation of viral genetic materials, as reported by Taylor et al., (1994).

The most advanced method utilised in up-to-date's genetical genomics, combining large microarray data sets with QTL results, gene function annotation and statistical analyses, is expression QTL (eQTL) analysis. Gene expression levels can be considered as quantitative traits, once their expression is heritable. If so, the information can be combined with marker data for the identification of loci influencing variation in the gene's expression. eQTL can be classified into cis- or trans-acting, located on the same or other genomic region of the regulated gene, respectively. The method allows for precise identification of candidate genes controlling complex traits and the expression of related genes, as well as for the construction of regulatory networks for these complex traits (Liu et al, 2006). By now, the application of eQTL method was conducted on yeasts, maize, mouse and human (Brem et al., 2002; Schadt et al., 2003; Page and Ruden, 2006; Shi C. et al., 2007).

Finally, laser capture microdissection (LCM) technology in combination with microarray approach is becoming an important tool for monitoring of transcript changes in specific types of plant cells isolated from heterogeneous tissues. The method allows precise and rapid isolation of pure cell populations from tissue of interest, while utilising laser beam and microscope to achieve transfer of the cells (Schnable et al., 2004). Regarding the fact that every cell has a unique transcriptome, metabolome, and proteome, the knowledge of the physiology of this basic unit of organism organization delivers substantial information about the whole plant. The usage of LCM in combination with cDNA microarrays to follow transcriptome profiles on a global scale in isolated phloem leaf cells in rice, captured epidermal and vascular cells in maize, apical and basal domains of *Arabidopsis* embryos and in mammalian cells to study prostate cancer were reported by Asano et al., (2002), Nakazano et al., (2003), Casson et al., (2005) and Rubin, (2001).

As a result of the genetic complexity of the examined traits, a simple answer to the question which particular gene or gene set could be involved in the creation of a given trait and in which manner it could regulate the pathways of interest is naturally not obvious and maybe even not predictable. However, given an ongoing improvement of microarray technology and continuous development of more precise and eligible methods for data analysis and validation, the future of array approach seems rather auspicious.

5 SUMMARY

Heterosis and virus resistance are agriculturally important phenomenons of maize. Hence, the knowledge about molecular background of these complex traits is of particular interest for breeding programs. The work presented in this thesis was conducted with the overall aim to study phenotypic and molecular events leading to hybrid vigour formation and SCMV resistance in maize. Technical objectives included: 1) investigation of plant height as model character to study heterosis under various experimental conditions and inbred-hybrid combinations, 2) identification of stages and plant parts explaining most of the plant height differences between inbreds and hybrids, 3) identification of differentially expressed genes in relation to heterosis, 4) determination of expression patterns between inbred parent-hybrid triplets in view of differing degrees of relatedness of triplets, 5) identification of differentially expressed genes in relation to SCMV resistance, 6) determination of expression patterns of virus infection over time, and 7) investigation of the potential and reliability of the combination of two expression profiling methods, such as microarrays and quantitative real-time PCR for candidate gene identification.

Plant materials included maize inbred lines: 002, 005 (European flints), 250 (Iowa Stiff Stalk dent), 301 (Lancaster dent), their inter-pool (002x301, 005x250, 250x002, 301x005) and intra-pool hybrids (002x005, 250x301) for hybrid vigour investigations, and four near-isogenic homozygous maize genotypes: highly susceptible F7 $^{SS/SS}$, fully resistant F7 $^{RR/RR}$, resistant at *Scmv1* on chromosome 6 F7 $^{SS/RR}$ and resistant at *Scmv2* on chromosome 3 F7 $^{RR/SS}$ for SCMV experiments.

Phenotypic study was carried out in order to determine which plant height components were crucial for the plant height difference between inbred lines and their hybrids. Plant height, number and length of internodes were measured in two year field and greenhouse experiments. Based on morphological analyses, apical meristem at terminal spikelet stage was chosen for further molecular investigations. Unigene microarrays were applied to investigate expression profiles in inbred lines and their hybrids, to identify candidate genes related to plant height heterosis and to investigate the mode of inheritance. Subsequently, quantitative RT-PCR method was employed to validate microarray data.

Virus resistance study was conducted with highly infective SCMV potyvirus. Time course experiment including mock inoculated plants was carried out under greenhouse conditions. Leaf samples were used for expression profiling analyses based on self-fabricated microarrays, containing selected maize expressed sequence tags and resistance gene analogs.

Similarly to the heterosis experiment, qRT-PCR was applied to confirm true differential expression of the identified genes.

Phenotypic study on hybrid vigour revealed more and longer internodes of hybrids as compared to parental inbreds. Furthermore, significantly higher hybrid plant height in the field versus greenhouse conditions was observed. This led to substantial mid-parent heterosis values for plant height, reaching 100.4 % and 150.3 % for lowermost internodes 8 and 9 in the field, as compared to 44.6 % and 59.7 % in the greenhouse. High-parent heterosis values reached 67.13 % and 89.94 % in the field, as compared to 27.23 % and 21.42 % in the greenhouse. In conclusion, length of lowermost internodes was the major factor differing between inbreds and hybrids, however no specific internode explaining most of the plant height heterosis across all triplets was defined.

Expression profiling experiment utilising unigene microarrays enabled identification of 433 significantly differentially expressed sequences in inbred versus hybrid comparisons, most of which up-regulated in the hybrid. Within this pool, 99 genes were identified to be common for at least two comparisons. Furthermore, higher number of significantly differentially expressed genes in related as compared to unrelated triplets was identified. Nevertheless, none of the genes was differentially expressed across all triplets. In addition, expression profiling data utilised to determine the mode of inheritance demonstrated the prevalence of overdominant gene action in the given combinations of genotypes. Subsequent application of quantitative RT-PCR method for microarray data validation approved true differential expression of two genes with homology to plant height, gibberellin-stimulated transcript 1-like rice protein and putative defensin. These two candidate genes seem to be directly or indirectly involved in the regulation of plant height heterosis in maize.

Time course expression profiling study of SCMV virus infection revealed significant differential expression of 65 sequences between four near isogenic lines differing at two introgressed *Scmv* QTL regions. Most of the genes were significantly differential for the F7 $^{SS/RR}$ subline, carrying *Scmv1* resistance allele on chromosome 6. Highest numbers of consistently differentially expressed genes were detected for time point before virus infection with 1 and with 12 hours after infection. Moreover, one fourth of the genes were significant at multiple time points. Thaumatin-like protein, alcohol dehydrogenase, and glutathione S-transferase identified in the array experiment, were previously reported to take part in pathogen infection response pathways. The majority of identified sequences were mapped to the regions outside the *Scmv1* and *Scmv2* QTLs. QRT-PCR applied to validate most

interesting sequences resulted in the confirmation of metallothionein-like protein, differentially expressed between resistant at both loci F7 $^{RR/RR}$ and resistant at *Scmv1* F7 $^{SS/RR}$.

Summarising, this study delivered a strong evidence for the different reaction of inbred lines to the environmental stress as compared to their hybrids, at the phenotype level. Moreover, the discrepancy in the architecture of inbred lines and the hybrids was revealed. These findings might be further exploited for the investigation of heterosis phenomenon at particular developmental stages, plant parts or tissue types.

6 ZUSAMMENFASSUNG

Heterosis und Virusresistenz sind landwirtschaftlich wichtige Eigenschaften beim Mais. Deshalb ist das Wissen über die molekularen Hintergründe dieser komplexen Merkmale für Züchtungsprogramme von besonderem Interesse. Die vorgestellte Arbeit hatte das grundsätzliche Ziel, die phänotypischen und molekularen Ereignisse, die zu Hybrid-Vitalität und SCMV Resistenz bei Mais führen, zu untersuchen. Die Studie umfasste: 1) die Messung der Wuchshöhe als Modellcharakter zur Untersuchung von Heterosis sowohl unter verschiedenen experimentellen Bedingungen als auch von mehreren Inzucht-Hybrid Kombinationen, 2) die Identifikation von Wachstums-Stadien und Pflanzenteilen, welche den größten Einfluss auf Unterschiede in der Wuchshöhe zwischen Inzuchtlinien und Hybriden geben, 3) die Identifikation von differentiell exprimierten Genen in Relation zu Heterosis, 4) die Bestimmung von Expressionsmustern zwischen geselbsteten Eltern-Hybrid Triplets im Hinblick auf unterschiedliche Grade der Verwandtschaft der Triplets, 5) die Identifikation von differentiell exprimierten Genen in Bezug auf SCMV Resistenz, 6) die Bestimmung von zeitlichen Expressionsmustern nach Virusinfektion, 7) die Untersuchung des Potentials und der Verlässlichkeit der Kombination von zwei Expressionsprofilierungs-Methoden, Mikroarrays und quantitativer realtime PCR (qRT-PCR) für die Kandidatengen-Identifikation.

Das Pflanzenmaterial umfasste die Inzuchtlinien 002, 005 (Europäische Flint-Linien), 250 (Iowa Stiff Stalk – Dent-Linien), 301 (Lancaster-Dent-Linien), deren Inter-pool- (002x301, 005x250, 250x002, 301x005) und Intrapoolhybriden (002x005, 250x301) für die Untersuchung der Hybrid-Vitalität. Für die SCMV Experimente wurden vier nah-isogene, homozygote Maisgenotypen genutzt: F7 $^{SS/SS}$ (hoch anfällig), F7 $^{SS/RR}$ (Träger des SCMV Resistenzgenes *Scmv1* auf Chromosom 6), F7 $^{RR/SS}$ (Träger des SCMV Resistenzgenes *Scmv2* auf Chromosom 3), sowie F7 $^{RR/RR.}$

Die phänotypischen Untersuchungen wurden durchgeführt, um zu bestimmen, welche Komponenten der Wuchshöhe für den Unterschied zwischen Inzuchtlinien und Hybriden bezüglich der Wuchshöhe essentiell sind. Die Wuchshöhe, die Anzahl und die Länge von Internodien wurden über zwei Jahre in Feld- und Gewächshausversuchen bestimmt. Basierend auf morphologischen Analysen, wurde das Apikalmeristem im „terminal spikelet“ - Stadium für weitere molekulare Untersuchungen ausgewählt. Unigene Mikroarrays wurden angewandt, um Expressionsprofile in Inzuchtlinien und Hybriden zu prüfen, um Kandidatengene für Wuchshöhenheterosis zu identifizieren und um die Art der Vererbung zu

erforschen. Anschließend wurde mittels der qRT-PCR Methode die Mikroarray-Daten validiert.

Bezüglich Virusresistenz wurde mit dem hochinfektiösen SCMV (Potyvirus) gearbeitet. Ein Zeitreihen - Experiment wurde unter Gewächshausbedingungen einschliesslich verschiedener Kontrollen durchgeführt. Für Expressionsprofilierungsstudien wurden Mikroarrays eigens hergestellt, die aufgrund von Vorstudien ausgewählte Mais Expressed Sequence Tags (ESTs) und Resistenzgenanaloga beinhalteten. Ähnlich dem Heterosis-Experiment, wurde die qRT-PCR angewandt um differentielle Expression der identifizierten Gene zu bestätigen.

Die phänotypischen Untersuchungen der Hybrid-Vitalität zeigten eine grössere Anzahl sowie längere Internodien bei den Hybriden im Vergleich zu den geselbsteten Eltern. Weiterhinwar die Heterosis für Wuchshöhe im Feld im Vergleich zum Gewächshaus signifikant erhöht. Die „mid parent“ Heterosis für Wuchshöhe überschritt 100% für die unteren Internodien im Feld, während sie im Gewächshaus <60 % war. Die Länge der unteren Internodien waren das Hauptunterscheidungsmerkmal zwischen Inzuchtlinien und Hybriden, obwohl kein bestimmtes Internodium gefunden wurde, welches den Hauptanteil der Wuchshöhen-Heterosis gemittelt über alle Triplets erklärte.

Die Expressionsprofilierungsexperimente, in denen „unigene“-Mikroarrays verwendet wurden, ermöglichten die Identifikation von 433 signifikant verschieden exprimierten Sequenzen in Vergleichen zwischen Inzuchtlinien und Hybriden, wobei in den meisten Fällen eine stärkere Expression in Hybriden vorlag. Es wurden 99 Gene identifiziert, die in mindestens zwei Vergleichen zwischen Triplets übereinstimmten. Außerdem wurde eine höhere Anzahl von signifikant verschieden exprimierten Genen in verwandten im Vergleich zu nicht-verwandten Triplets gefunden. Dennoch wurde kein Gen in allen Triplets differentiell zwischen Inzuchtlinien und Hybriden exprimiert.

Die Ergebnisse der Expressionsprofilierungsexperimente wiesen eine Prävalenz für überdominante Genaktivität in den untersuchten Genotyp-Kombinationen hin. Eine anschließende Anwendung der qRT-PCR - Methode für eine Absicherung der Mikroarray-Daten bestätigte eine differentielle Expression von zwei Genen mit potentiell direktem Einfluss auf Wuchshöhe: ein mutmasslich Gibberellin-stimuliertes Transkript mit Homologie zu einem entsprechenden Reisgen sowie ein Defensingen. Diese zwei Kandidatengene scheinen direkt oder indirekt in die Regulation von Wuchshöhen-Heterosis bei Mais involviert zu sein.

Ein Zeitreihenexperiment zur Expressionprofilierung in Zusammenhang mit SCMV-Resistenz offenbartefür 65 Sequenzen eine signifikant verschiedene Expression zwischen nah isogenen

Linien, welche sich an zwei eingelagerten *Scmv*-QTL Regionen unterschieden. Die meisten der Gene waren stärker exprimiert in F7 $^{SS/RR}$, die das *Scmv1* Resistenzallel auf Chromosom 6 trägt. Die höchste Anzahl differentiell exprimierter Gene wurde zwischen dem Zeitpunkt unmittelbar vor der Virusinfektion und zwölf Stunden nach der Infektion gefunden. Ein Viertel der 65 Gene war an mehreren Zeitpunkte differentiell exprimiert. Thaumatin-ähnliche Proteine, eine Alkohol-Dehydrogenase und eine Glutathione S-Transferase, die in diesem Experiment identifiziert wurden, sind in anderen Pathosystemen an der Infektionsantwort infizierter Wirtspflanzen beteiligt. Die Mehrzahl der identifizierten Sequenzen wurde in Regionen außerhalb der *Scmv1* und *Scmv2* QTL kartiert. Mittels qRT-PCR wurde die differentielle Expression zwischen F7 $^{RR/RR}$ und F7 $^{SS/RR}$ für ein Gen bestätigt, das ein Metallothionein-ähnliches Protein exprimiert.

Zusammenfassend lieferte diese Arbeit einen Beleg für die unterschiedliche, phänotypische Reaktion von Inzuchtlinien auf Umweltstress im Vergleich zu deren Hybriden. Des Weiteren wurden wesentliche Faktoren für den Unterschied in der Architektur der hier untersuchten Inzuchtlinien und deren Hybriden erkannt. Diese Ergebnisse könnten durch Untersuchungen in bestimmten Entwicklungsstadien, Pflanzenteilen oder Gewebetypen zur Untersuchung des Heterosis-Phänomens weiter ausgedehnt werden.

Zusätzlich erlaubte die molekulare Untersuchung dieser komplex vererbten Merkmale mit Mikroarray Technologie die Identifikation von einzelnen Genen und Genexpressionsmustern, die in Zusammenhang mit dem analysierten Phänomen stehen. Zeitgleiches filtern von Tausenden von Genen in einem einzigen Experiment scheint vielversprechend im Hinblick auf eine wesentliche Reduzierung von Kosten und Arbeitszeit, welche für eine Entwicklung von Inzuchtlinien für erfolgreiche Zuchtprogramme notwendig sind. Eine nachfolgende Verbindung des Mikroarry-Ansatzes mit der qRT-PCR Methode demonstrierte das Potential und die Verlässlichkeit der Kombination dieser zwei Techniken bezüglich der Identifikation von Kandidatengenen.

7 REFERENCES

Abler, B.S.B., Edwards, M.D., Stuber, C.W. (1991). Isoenzymatic identification of quantitative trait loci in crosses of elite maize inbreds. Crop Science 31: 267-274.

Abruzzo, L.V., Lee, K.Y., Fuller, A., Silverman, A., Keating, M.J., Medeiros, L.J., Coombes, K.R. (2005). Validation of oligonucleotide microarray data using microfluidic low-density arrays: a new statistical methos to normalize real-time RT-PCR data. BioTechniques 38: 785-792.

Aharoni, A., Keizer, L.C.P., Bouwmeester, H.J., Sun, Z.K., Alvarez-Huerta, M., Verhoeven, H.A., Blaas, J., van Houwelingen, A.M.M.L., De Vos, R.C.H., van der Voet, H., Jansen, R.C., Guis, M., Mol, J., Davis, R.W., Schena, M., van Tunen, A.J., O'Connell, A.P. (2000). Identification of the SAAT gene involved in strawberry flavor biogenesis by use of DNA microarrays. Plant Cell 12: 647-661.

Ahmadi, N., Albar, L., Pressoir, G., Pinel, A., Fargette, D., Ghesquiere, A. (2001). Genetic basis and mapping of the resistance to rice yellow mottle virus. III. Analysis of QTL efficiency in introgressed progenies confirmed the hypothesis of complementary epistasis between two resistance QTLs. Theoretical and Applied Genetics 103: 1084-1092.

Ajmone-Marsan, P., Livini, C., Messmer, M.M., Melchinger, A.E., Motto, M. (1992). Cluster analysis of RFLP data from related maize inbred lines of the BSSS and LSC heterotic groups and comparison with pedigree data. Euphytica 60: 139-148.

Allison, D.B., Page, G.P., Beasley, T.M., Edwards, J.W.: DNA microarrays and related genomics techniques, design, analysis and interpretation of experiments. Chapman & Hall/CRC, Boca, Raton, London, New York (2006).

Arru, L., Faccini, N., Govoni, C., Cattivelli, L., Pecchioni, N., Delogu, G., Stanca, A.M., Vale, G. (2003). The PCR-based marker MWG2018 linked to the *RDG2A* leaf stripe resistance gene is a useful tool for assessing barley resistance in breeding programs. Crop Science 43: 1036-1042.

Asano, T., Masumura, T., Kusano, H., Kikuchi, S., Kurita, A., Shimada, H., Kadowaki, K. (2002). Construction of a specialized cDNA library from plant cells isolated by laser capture microdissection: toward comprehensive analysis of the genes expressed in the rice phloem. Plant Journal 32: 401-408.

Ashikari, M., Wu, J.Z., Yano, M., Sasaki, T., Yoshimura, A. (1999). Rice gibberellin-insensitive dwarf mutant gene *Dwarf 1* encodes the alpha-subunit of GTP-binding protein. Proceedings of the National Academy of Sciences of the United States of America 96: 10284-10289.

Auger, D.L., Gray, A.D., Ream, T.S., Kato, A., Coe, E.H., Birchler, J.A. (2005). Nonadditive gene expression in diploid and triploid hybrids of maize. Genetics 169: 389-397.

Bajaj, Y.P.S.: Biotechnology in maize improvement. In: Bajaj, Y.P.S. (ed) Biotechnology in agriculture and forestry 25 Maize, pp. 3-23. Springer-Verlag, Berlin, Heidelberg, New York, London, Paris, Tokyo, Hong Kong, Barcelona, Budapest (1994).

Barnett, O.W. (1991). *Potyviridae*, a proposed family of plant-viruses. Archives of Virology 118: 139-141.

Barone, A.: Molecular marker-assisted selection for resistance to pathogens in tomato. Marker assisted selection: a fast track to increase genetic gain in plant and animal breeding?, Turin, Italy (2003).

Bartual, R., Carbonell, E.A., Marsal, J.I., Tello, J.C., Campos, T. (1991). Gene-action in the resistance of peppers (Capsicum-Annuum) to phytophthora stem blight (*Phytophthora-Capsici* L.). Euphytica 54: 195-200.

Baulcombe, D. (2004). RNA silencing in plants. Nature 431: 356-363.

Beadle, G.W. (1939). Teosinte and the origin of maize. Journal of Heredity 30: 245-247.

Beadle, G.W.: Teosinte and the origin of maize. In: Walden, D.B. (ed) Maize breeding and genetics, pp. 113-128. John Wiley & Sons, New York (1978).

Beadle, G.W. (1980). Ancestry of corn. Scientific American 242: 112-119.

Beavis, W.D., Keim, P.: Identification of QTL that are affected by environment. In: Kang, M., Gauch, H. (eds) Genotype by environment interactions, pp. 123-149. CRC Press, Boca Raton (1995).

Becker, H.C.: Pflanzenzüchtung. Ulmer Verlag, Stuttgart (1993).

Benchimol, L.L., L., D.S.J.C., Garcia, A.A.F., Kono, P.M.S., Mangolin, C.A., Barbosa, A.M.M., Coelho, A.S.G., De Souza, A.P. (2000). Genetic diversity in tropical maize inbred lines: heterotic group assignment and hybrid determined by RFLP markers. Plant Breeding 119: 491-496.

Benjamini, Y., Hochberg, Y. (1995). Controlling the false discovery rate - a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B-Methodological 57: 289-300.

Bennett, M.D.: The spacial distribution of chromosomes. Kew Chromosome Conference II, pp. 71-79, London, George Allen and Unwin (1983).

Betrán, F.J., Ribaut, J.M., Beck, D., de León, D.G. (2003). Genetic diversity, specific combining ability, and heterosis in tropical maize under stress and nonstress environments. Crop Science 43: 797-806.

Birchler, J.A., Auger, D.L., Riddle, N.C. (2003). In search of molecular basis of heterosis. Plant Cell 15: 2236-2239.

Bowtell, D.D.L. (1999). Options available - from start to finish - for obtaining expression data by microarray. Nature Genetics 21: 25-32.

Brem, R.B., Yvert, G., Clinton, R., Kruglyak, L. (2002). Genetic dissection of transcriptional regulation in budding yeast. Science 296: 752-755.

Bruce, A.B. (1910). The Mendelian theory of heredity and the augmentation of vigour. Science 32: 627-628.

Brunner, A.M., Yakovlev, I.A., Strauss, S. (2004). Validating internal controls for quantitative plant gene expression studies. BMC Plant Biology 4: 14.

Caranta, C., Palloix, A., Lefebvre, V., Daubeze, A.M. (1997). QTLs for a component of partial resistance to cucumber mosaic virus in pepper: restriction of virus installation in host-cells. Theoretical and Applied Genetics 94: 431-438.

Carrington, J.C., Kasschau, K.D., Mahajan, S.K., Schaad, M.C. (1996). Cell-to-cell and long-distance transport of viruses in plants. Plant Cell 8: 1669-1681.

Casati, P., Walbot, V. (2003). Gene expression profiling in response to ultraviolet radiation in maize genotypes with varying flavonoid content. Plant Physiology 132: 1739-1754.

Casson, S., Spencer, M., Walker, K., Lindsey, K. (2005). Laser capture microdissection for the analysis of gene expression during embryogenesis of *Arabidopsis*. Plant Journal 42: 111-123.

Charpentier, L.J. (1956). Systemic insecticide studies for control of vectors and sugarcane mosaic in Louisiana. Journal of Economic Entomology 49: 413-414.

Chen, W.Q.J., Chang, S.H., Hudson, M.E., Kwan, W.K., Li, J.Q., Estes, B., Knoll, D., Shi, L., Zhu, T. (2005). Contribution of transcriptional regulation to natural variations in *Arabidopsis*. Genome Biology 6: R32.

Chen, Z.J., Wang, J.L., Tian, L., Lee, H.S., Wang, J.Y.J., Chen, M., Lee, J.S.J., Josefsson, C., Madlung, A., Watson, B., Lippman, Z., Vaughn, M., Pires, J.C., Colot, V., Doerge, R.W., Martienssen, R.A., Comai, L., Osborn, T.C. (2004). The development of an *Arabidopsis* model system for genome-wide analysis of polyploidy effects. Biological Journal of the Linnean Society 82: 689-700.

Chuang, C.F., Meyerowitz, E.M. (2000). Specific and heritable genetic interference by double-stranded RNA in *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences of the United States of America 97: 4985-4990.

Churchill, G.A. (2002). Fundamentals of experimental design for cDNA microarrays. Nature Genetics 32: 490-495.

Cigan, A.M., Unger-Wallace, E., Haug-Collet, K. (2005). Transcriptional gene silencing as a tool for uncovering gene function in maize. Plant Journal 43: 929-940.

Collins, N.C., Webb, C.A., Seah, S., Ellis, J.G., Hulbert, S.H., Pryor, A. (1998). The isolation and mapping of disease resistance gene analogs in maize. Molecular Plant-Microbe Interactions 11: 968-978.

Comings, D.E., MacMurray, J.P. (2000). Molecular heterosis: a review. Molecular Genetics and Metabolism 71: 19-31.

Comstock, J.C., Lentini, R.S.: Sugarcane mosaic virus disease pp. SS-AGR-209. Institute of Food and Agricultural Sciences, University of Florida, Florida (2002).

Creelman, R.A., Mullet, J.E. (1997). Biosynthesis and action of jasmonates in plants. Annual Review of Plant Physiology and Plant Molecular Biology 48: 355-381.

Cregan, P.B., Mudge, J., Fickus, E.W., Danesh, D., Denny, R., Young, N.D. (2000). Two simple sequence repeat markers to select for soybean cyst nematode resistance conditioned by the *rhg1* locus. Theoretical and Applied Genetetics 99: 811-818.

Crow, J.F. (1948). Alternative hypotheses of hybrid vigour. Genetics 33: 477-487.

Crute, I.R., Pink, D.A.C. (1996). Genetics and utilization of pathogen resistance in plants. Plant Cell 8: 1747-1755.

Cruz, S.S. (1999). Perspective: phloem transport of viruses and macromolecules - what goes in must come out. Trends in Microbiology 7: 237-241.

Czechowski, T., Bari, R.P., Stitt, M., Scheible, W.R., Udvardi, M.K. (2004). Real-time RT-PCR profiling of over 1400 *Arabidopsis* transcription factors: unprecedented sensitivity reveals novel root- and shoot-specific genes. Plant Journal 38: 366-379.

Dallas, P.B., Gottardo, N.G., Firth, M.J., Beesley, A.H., Hoffmann, K., Terry, P.A., Freitas, J.R., Boag, J.M., Cummings, A.J., Kees, U.R. (2005). Gene expression levels assessed by oligonucleotide microarray analysis and quantitative real-time RT-PCR - how well do they correlate? BMC Genomics 6: 59.

Davenport, C.B. (1908). Degeneration, albinism and inbreeding. Science 28: 454-455.

Davey, M.R., Cocking, E.C., Freeman, J., Pearce, N., Tudor, I. (1980). Transformation of petunia protoplasts by isolated *Agrobacterium* plasmids. Plant Science Letters 18: 307-313.

Davierwala, A.P., Reddy, A.P.K., Lagu, M.D., Ranjekar, P.K., Gupta, V.S. (2001). Marker assisted selection of bacterial blight resistance genes in rice. Biochemical Genetics 39: 261-278.

de Torres, M., Sanchez, P., Fernandez-Delmond, I., Grant, M. (2003). Expression profiling of the host response to bacterial infection: the transition from basal to induced defence responses in RPM1-mediated resistance. Plant Journal 33: 665-676.

Desprez, T., Amselem, J., Caboche, M., Höfte, H. (1998). Differential gene expression in *Arabidopsis* monitored using cDNA arrays. Plant Journal 14: 643-652.

Dhillon, B.S., Boppenmaier, J., Pollmer, W.G., Herrmann, R.G., Melchinger, A.E. (1993). Relationship of restriction-fragment-length-polymorphisms among European maize inbreds with ear dry-matter yield of their hybrids. Maydica 38: 245-248.

Dilger, M., Felsenstein, F.G., Schwarz, G. (2003). Identification and quantitative expression analysis of genes that are differentially expressed during conidial germination in *Pyrenophora teres*. Molecular Genetics and Genomics 270: 147-155.

Dowswell, C.D., Paliwal, R.L., Cantrell, R.P.: Maize in the third world. Westview Press, Boulder (1996).

Dubreuil, P., Dufour, P., Krejci, E., Causse, M., deVienne, D., Gallais, A., Charcosset, A. (1996). Organization of RFLP diversity among inbred lines of maize representing the most significant heterotic groups. Crop Science 36: 790-799.

Dudley, J.W., Maroof, M.A.S., Rufener, G.K. (1991). Molecular markers and grouping of parents in maize breeding programs. Crop Science 31: 718-723.

Dudley, J.W. (1993). Molecular markers in plant improvement - manipulation of genes affecting quantitative traits. Crop Science 33: 660-668.

Dussle, C.M., Melchinger, A.E., Kuntze, L., Stork, A., Lubberstedt, T. (2000). Molecular mapping and gene action of *Scm1* and *Scm2*, two major QTL contributing to SCMV resistance in maize. Plant Breeding 119: 299-303.

Dussle, C.M.: Development and fine mapping of markers closely linked to the SCMV resistance loci *Scmv1* and *Scmv2* in European maize (*Zea mays* L.) Angewandte Genetik und Pflanzenzüchtung, pp. 80. University of Hohenheim, Hohenheim (2002).

East, E.M.: Inbreeding in corn. Connecticut Agricultural Experimental Station Report, 1907-1908, pp. 419-428 (1908).

Eckardt, N.A. (2003). Maize genetics 2003. Plant Cell 15: 1053-1055.

Edwards, M.D., Stuber, C.W., Wendel, J.F. (1987). Molecular-marker-facilitated investigations of quantitative-trait loci in maize. 1. Numbers, genomic distribution and types of gene-action. Genetics 116: 113-125.

Feng, C.P., Mundy, J. (2006). Gene discovery and functional analyses in the model plant *Arabidopsis*. Journal of Integrative Plant Biology 48: 5-14.

Fernandes, J., Brendel, V., Gai, X.W., Lal, S., Chandler, V.L., Elumalai, P., Galbraith, D.W., Pierson, E.A., Walbot, V. (2002). Comparison of RNA expression profiles based on maize expressed sequence tag frequency analysis and micro-array hybridization. Plant Physiology 128: 896-910.

Findley, W.R., Louie, R., Knoke, J.K.: Breeding corn for resistance to corn viruses in Ohio. In: Wilkinson, D.J. (ed) Proceedings of annual corn sorghum research conference pp. 52-67. American Seed Trade Association, Washington (1984).

Francia, E., Tacconi, G., Crosatti, C., Barabaschi, D., Bulgarelli, D., Dall'Aglio, E., Vale, G. (2005). Marker assisted selection in crop plants. Plant Cell Tissue and Organ Culture 82: 317-342.

Fuchs, E., Gruntzig, M. (1995). Influence of sugarcane mosaic-virus (SCMV) and maize-dwarf mosaic-virus (MDMV) on the growth and yield of 2 maize varieties. Journal of Plant Diseases and Protection 102: 44-50.

Galinat, W.C.: The patterns of plant structures in maize. In: FReeling, M., Walbot, V. (eds) The maize handbook, pp. 61-65. Springer-Verlag, New York, Berlin (1994).

Galinat, W.C.: The origin of corn. In: Spraque, G.F., Dudley, J.W. (eds) Corn and corn improvement, pp. 1-31. ASA-CSSA-SSSA, Madison (1988).

Gepts, P. (2002). A comparison between crop domestication, classical plant breeding, and genetic engineering. Crop Science 42: 1780-1790.

Geraghty, D., Peifer, M.A., Rubenstein, I., Messing, J. (1981). The primary structure of a plant-storage protein - zein. Nucleic Acids Research 9: 5163-5174.

Ginzinger, D.G. (2002). Gene quantification using real-time quantitative PCR: an emerging technology hits the mainstream. Experimental Hematology 30: 503-512.

Gregersen, P.L., Brinch-Pedersen, H., Holm, P.B. (2005). A microarray based, comparative analysis of the gene expression profiles during grain development in transgenic and wild type wheat. Transgenic Research 14: 887-905.

Grumet, R., Kabelka, E., McQueen, S., Wai, T., Humphrey, R. (2000). Characterization of sources of resistance to the watermelon strain of Papaya ringspot virus in cucumber: allelism and co-segregation with other potyvirus resistances. Theoretical and Applied Genetics 101: 463-472.

Guo, M., Rupe, M.A., Zinselmeier, C., Habben, J., Bowen, B.A., Smith, O.S. (2004). Allelic variation of gene expression in maize hybrids. Plant Cell 16: 1707-1716.

Guo, M., Rupe, M.A., Yang, X.F., Crasta, O., Zinselmeier, C., Smith, O.S., Bowen, B. (2006). Genome-wide transcript analysis of maize hybrids: allelic additive gene expression and yield heterosis. Theoretical and Applied Genetics 113: 831-845.

Hallauer, A.R.: Temperate maize and heterosis. In: Coors, J.G., Pandey, S. (eds) The genetics and exploitation of heterosis in corps, pp. 353-360. ASA-CSSA-SSSA, Madison (1999).

Han, F., Romagosa, I., Ullrich, S.E., Jones, B.L., Hayes, P.M., Wesenberg, D.M. (1997). Molecular marker-assisted selection for malting quality traits in barley. Molecular Breeding 3: 427-437.

Harberd, N.P., Freeling, M. (1989). Genetics of dominant gibberellin-insensitive dwarfism in maize. Genetics 121: 827-838.

Harlan, J.R., Chapman, G.P.: Origins and processes of domestication. In: Harlan, J.R., Chapman, G.P. (eds) Grass Evolution and Domestication, pp. 159-175. Cambridge University Press, Cambridge (1992).

Hämäläinen, J.H., Watanabe, K.N., Valkonen, J.P.T., Arihara, A., Plaisted, R.L., Pehu, E., Miller, L., Slack, S.A. (1997). Mapping and marker-assisted selection for a gene for extreme resistance to potato virus Y. Theoretical and Applied Genetics 94: 192-197.

Helentjaris, T., Heun, M.: Analysis of traits with complex inheritance in maize using molecular markers. In: Freeling, M., Walbot, V. (eds) The maize handbook, pp. 509-513. Springer-Verlag, New York, Berlin, Heidelberg, London, Paris, Tokyo, Hong Kong, Barcelona, Budapest (1994).

Helliwell, C.A., Chin-Atkins, A.N., Wilson, I.W., Chapple, R., Dennis, E.S., Chaudhury, A. (2001). The *Arabidopsis AMP1* gene encodes a putative glutamate carboxypeptidase. Plant Cell 13: 2115-2125.

Hepting, L. (1978). Analysis of a 7x7-variety cross diallel in relation to choosing base materials for breeding of hybrid varieties in rye. Journal of Plant Breeding 80: 188-197.

Herrmann, M. (2007). A diallel analysis of various traits in winter triticale. Plant Breeding 126: 19-23.

Hieter, P., Boguski, M. (1997). Functional genomics: it's all how you read it. Science 278: 601-602.

Higuchi, R., Fockler, R., Dollinger, G., Watson, R. (1993). Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. Biotechnology 11: 1026-1030.

Hoffmann, T., Kalinowski, G., Schwab, W. (2006). RNAi-induced silencing of gene expression in strawberry fruit (*Fragaria x ananassa*) by agroinfiltration: a rapid assay for gene function analysis. Plant Journal 48: 818-826.

Hooykaas, P.J.J. (1989). Transformation of plant-cells via *Agrobacterium*. Plant Molecular Biology 13: 327-336.

Horvath, D.P., Schaffer, R., West, M., Wisman, E. (2003). *Arabidopsis* microarrays identify conserved and differentially expressed genes involved in shoot growth and development from distantly related plant species. Plant Journal 34: 125-134.

Howes, N.K., Woods, S.M., Townley-Smith, T.F. (1998). Simulations and practical problems of applying multiple marker assisted selection and doubled haploids to wheat breeding programs. Euphytica 100: 225-230.

Hull, F.H. (1945). Recurrent selection for specific combining ability in corn. Journal of American Society of Agronomy 37: 134-145.

Jacobs, T. (1997). Why do plant cells divide. Plant Cell 9: 1021-1029.

Jain, K.K.: Biochips and microarrays. Technology and commercial potential. Informa Publishing Group Ltd, London (2000).

Jain, R.K., Rao, G.P., Varma, A.: Present status of management of sugarcane mosaic virus. In: Hadidi, A., Khetarpal, R.K., Koganezava, H. (eds) Plant virus disease control, pp. 495-523. American Publishing Company, Minnesota (1998).

Jenson, S.D., Robetorye, R.S., Bohling, S.D., Schumacher, J.A., Morgan, J.W., Lim, M.S., Elenitoba-Jonson, K.S.J. (2003). Validation of cDNA microarray gene expression data obtained from linearly amplified RNA. Journal of Clinical Pathology: Molecular Pathology 56: 307-312.

Jiang, J.X., Zhou, X.P. (2002). Maize dwarf mosaic disease in different regions of China is caused by sugarcane mosaic virus. Archives of Virology 147: 2437-2443.

Jones, D.F. (1917). Dominance of linked factors as a means of accounting for heterosis. Genetics 2: 466-479.

Kang, B.C., Yeam, I., Jahn, M.M. (2005). Genetics of plant virus resistance. Annual Review of Phytopathology 43: 581-621.

Kauffmann, K.D., Crum, C.W., Lindsey, M.F. (1982). Exotic germplasm in a corn breeding program. III. Corn Breeder's School 18: 6-39.

Keeble, F., Pellew, C. (1910). The mode of inheritance of stature and time of flowering in peas (*Pisum sativum*). Journal of Genetics 1: 47-56.

Keller, B., Emrich, K., Hoecker, N., Sauer, M., Hochholdinger, F., Piepho, H.-P. (2005). Designing a microarray experiment to estimate dominance in maize (*Zea mays* L.). Theoretical and Applied Genetics 111: 57-64.

Kerr, M.K., Martin, M., Churchill, G.A. (2000). Analysis of variance for gene expression microarray data. Journal of Computational Biology 7: 819-837.

Kerr, M.K., Churchill, G.A. (2001). Experimental design for gene expession micorarrays. Biostatistics 2: 183-201.

Kiesselbach, T.A. (1922). Corn investigations. Bulletin of the Agricultural Experiment Station of Nebraska 20: 5-151.

Kiesselbach, T.A.: The structure and reproduction of corn. In: Kiesselbach, T.A. (ed) The structure and reproduction of corn. 50th anniversary edition, pp. 3-95. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1949).

Kim, B.R., Nam, H.Y., Kim, S.U., Kim, S.I., Chang, Y.J. (2003). Normalization of reverse transcription quantitative-PCR with housekeeping genes in rice. Biotechnology Letters 25: 1869-1872.

Klein, T.M., Wolf, E.D., Wu, R., Sanford, J.C. (1987). High-velocity microprojectiles for delivering nucleic-acids into living cells. Nature 327: 70-73.

Knapp, S.J.: Mapping quantitative trait loci. In: Phillips, R.L., Vasil, I.K. (eds) DNA-based markers in plants, pp. 58-96. Kluwer Academic Publishers, Dordrecht (1994).

Knapp, S.J. (1998). Marker-assisted selection as a strategy for increasing the probability of selecting superior genotypes. Crop Science 38: 1164-1174.

Kohane, I.S., Kho, A.T., Butte, A.J.: Microarrays for an integrative genomics. A Brandford Book, The MIT Press, Cambridge, Massachusetts, London (2003).

Konarev, V.G.: Physiological and biochemical aspects of heterosis. In: Jánossy, A., Lupton, F.G.H. (eds) Heterosis in plant breeding. Proceedings of the seventh congress of EUCARPIA, pp. 81-93. Elsevier Scentific Publishing Company and Akadémiai Kiadó, Budapest (1976).

Kovács, G., Gaborjanyi, R., Vasdinyei, R., Toldi, E. (1998). Resistance of maize inbred lines to maize dwarf mosaic and sugarcane mosaic potyviruses. Cereal Research Communications 26: 195-201.

Krens, F.A., Molendijk, L., Wullems, G.J., Schilperoort, R.A. (1982). Invitro transformation of plant-protoplasts with Ti-plasmid DNA. Nature 296: 72-74.

Kuntze, L., Fuchs, E., Gruntzig, M., Schulz, B., Klein, D., Melchinger, A.E. (1997). Resistance of early-maturing European maize germplasm to sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV). Plant Breeding 116: 499-501.

Kusaba, M., Miyahara, K., Iida, S., Fukuoka, H., Takano, T., Sassa, H., Nishimura, M., Nishio, T. (2003). Low glutelin content1: a dominant mutation that suppresses the glutelin multigene family via RNA silencing in rice. Plant Cell 15: 1455-1467.

Kusaba, M. (2004). RNA interference in crop plants. Current Opinion in Biotechnology 15: 139-143.

Lacape, J.M., Nguyen, T.B., Hau, B., Giband, M.: Targetted introgression of cotton fiber quality QTLs using molecular markers. Marker assisted selection: a fast track to increase genetic gain in plant and animal breeding?, Turin, Italy (2003).

Lamb, B.C.: The applied genetics of plants, animals, humans and fungi. Imperial College Press, London (2000).

Lamkey, K.R., Edwards, J.W.: Guantitative genetics of heterosis. In: Coors, J.G., Pandey, S. (eds) The genetics and exploitation of heterosis in crops, pp. 31-48. ASA-CSSA-SSSA, Madison (1998).

Lancashire, D.P., Bleiholder, H., Van den Boom, T., Langelüddeke, P., Stauss, R., Weber, E., Witzenberger, A. (1991). A uniformal decimal code for growth stages of crops and weeds. Annals of Applied Biology 119: 561-601.

Lanza, L.L.B., deSouza, C.L., Ottoboni, L.M.M., Vieira, M.L.C., deSouza, A.P. (1997). Genetic distance of inbred lines and prediction of maize single-cross performance using RAPD markers. Theoretical and Applied Genetics 94: 1023-1030.

Law, M.D., Moyer, J.W., Payne, G.A. (1989). Effect of host-resistance on pathogenesis of maize-dwarf mosaic-virus. Phytopathology 79: 757-761.

Lee, E.-J., Koizumi, N., Sano, H. (2004). Identification of genes that are up-regulated in concern during sugar depleition in *Arabidopsis*. Plant Cell and Environment 27: 337-345.

Lee, H.S., Wang, J.L., Tian, L., Jiang, H.M., Black, M.A., Madlung, A., Watson, B., Lukens, L., Pires, J.C., Wang, J.J., Comai, L., Osborn, T.C., Doerge, R.W., Chen, Z.J. (2004). Sensitivity of 70-mer oligonucleotides and cDNAs for microarray analysis of gene expression in *Arabidopsis* and its related species. Plant Biotechnology Journal 2: 45-57.

Lee, J.-M. (1995). DNA markers and plant breeding programs. Advances in Agronomy 55: 265-341.

Lee, J.-M., Williams, M.E., Tingey, S.V., Rafalski, J.A. (2002). DNA array profiling of gene expression changes during maize embryo development. Functional & Integrative Genomics 2: 13-27.

Lee, M., Godshalk, E.B., Lamkey, K.R., Woodman, W.W. (1989). Association of restriction fragment length polymorphisms among maize inbreds with agronomic performance of their crosses. Crop Science 29: 1067-1071.

Lee, M.L.T., Kuo, F.C., Whitmore, G.A., Sklar, J. (2000). Importance of replication in microarray gene expression studies: Statistical methods and evidence from repetitive cDNA hybridizations. Proceedings of the National Academy of Sciences of the United States of America 97: 9834-9839.

Lei, J.D., Agrios, G.N. (1986). Detection of virus-infection and spread by immunofluorescent staining of enzyme treated leaves. Phytopathology 76: 1031-1033.

Leonardi, A., Damerval, C., Hebert, Y., Gallais, A., Devienne, D. (1991). Association of protein amount polymorphism (Pap) among maize lines with performances of their hybrids. Theoretical and Applied Genetics 82: 552-560.

Li, J., Pankratz, M., Johnson, J.A. (2002). Differential gene expression patterns revealed by oligonucleotide versus long cDNA arrays. Toxicological Sciences 69: 383-390.

Li, Z.-K., Luo, J.L., Wang, D.L., Shu, Q.Y., Tabien, R., Zhong, D.B., Ying, C.S., Stansel, J.W., Khush, G.S., Paterson, A.H. (2001). Overdominant epistatic loci are the primary genetic basis of inbreeding derpession and heterosis in rice. I. Biomass and grain yield. Genetics 158: 1737-1753.

Lincoln, T., Zeiger, E.: Plant Physiology. Sinauer, Sunderland (1998).

Lindbo, J.A., Silva-Rosales, L., Proebsting, W.M., Dougherty, W.G. (1993). Induction of a highly specific antiviral state in transgenic plants: implications for regulation of gene expression and virus resistance. Plant Cell 5: 1749-1759.

Liu, X.Y., Xie, Z.M., Chen, H.Z. (2006). Advance in genetical genomics. Progress in Biochemistry and Biophysics 33: 1030-1034.

Longley, A.E. (1941) a. Chromosome morphology in maize and its relatives. Botanical Review 7: 263-289.

Longley, A.E. (1941) b. Knob positions on teosinte chromosomes. Journal of Agricultural Research 62: 0401-0413.

Louie, R., Findley, W.R., Knoke, J.K., Mcmullen, M.D. (1991). Genetic-basis of resistance in maize to 5 maize-dwarf mosaic-virus strains. Crop Science 31: 14-18.

Lübberstedt, T., Melchinger, A.E., Schön, C., Utz, H.F., Klein, D. (1997). QTL mapping in testcrosses of European flint lines of maize: I. Comparison of different testers for forage yield traits. Crop Science 37: 921-931.

Lübberstedt, T. (1998). Comparative QTL mapping of resistance to *Ustilago maydis* across four populations of European flint-maize. Theoretical and Applied Genetics 97: 1321-1330.

Lübberstedt, T., Melchinger, A.E., Fähr, S., Klein, D., Dally, A., Westhoff, P. (1998). QTL mapping in testcrosses of flint lines of maize: III. Comparison across populations for forage traits. Crop Science 38: 1278-1289.

Lübberstedt, T., Mohler, V., Wenzel, G. (2002). Function of genetic material: Genes involved in quantitative and qualitative resistance. Genetics 63: 80-106.

Lübberstedt, T., Ingvardsen, C., Melchinger, A.E., Xing, Y., Salomon, R., Redinbaugh, M.G. (2006). Two chromosome segments confer multiple potyvirus resistance in maize. Plant Breeding 125: 352-356.

Luo, J.L., Li, Z.-K., Mei, H.W., Shu, Q.Y., Tabien, R., Zhong, D.B., Ying, C.S., Stansel, J.W., Khush, G.S., Paterson, A.H. (2001). Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. II. Grain yiels components. Genetics 158: 1755-1771.

Ma, J., Morrow, D.J., Fernandes, J., Walbot, V. (2006). Comparative profiling of the sense and antisense transcriptome of maize lines. Genome Biology 7: R22.

Maguire, T.L., Grimmond, S., Forrest, A., Iturbe-Ormaetxe, I., Meksem, K., Gresshoff, P. (2002). Tissue-specific gene expression in soybean (Glycine max) detected by cDNA microarray analysis. Journal of Plant Physiology 159: 1361-1374.

Maiti, R., Wesche-Ebeling, P.: Origin, evolution, and domestication of maize: recent approach. In: Mati, R., Wesche-Ebeling, P. (eds) Maize science, pp. 1-36. Science Publishers, Inc., Enfield (1998).

Mangelsdorf, P.C. (1961). Introgression in maize. Euphytica 10: 157-168.

Marshall, E. (2004). Getting the noise out of gene arrays. Science 306: 630-631.

Martinez, M.J., Aragon, A.D., Rodriguez, A.L., Weber, J.M., Timlin, J.A., Sinclair, M.B., Haaland, D.M., Werner-Washburne, M. (2003). Identification and removal of contaminating fluorescence from commercial and in-house printed DNA microarrays. Nucleic Acids Research 31: e18.

McKern, N.M., Shukla, D.D., Toler, R.W., Jensen, S.G., Tosic, M., Ford, R.E., Leon, O., Ward, C.W. (1991). Confirmation that the sugarcane mosaic-virus subgroup consists of 4 distinct potyviruses by using peptide profiles of coat proteins. Phytopathology 81: 1025-1029.

McManus, M.T., White, D.W.R., McGregor, P.G. (1994). Accumulation of a chymotrypsin inhibitor in transgenic tobacco can affect the growth of insect pests. Transgenic Research 3: 50-58.

McMullen, M.D., Louie, R. (1989). The linkage of molecular markers to a gene controlling the symptom response in maize to maize-dwarf mosaic-virus. Molecular Plant-Microbe Interactions 2: 309-314.

Melchinger, A.E., Lee, M., Lamkey, K.R., Hallauer, A.R., Woodman, W.L. (1990). Genetic diversity for restriction-fragment-length-polymorphisms and heterosis for 2 diallel sets of maize inbreds. Theoretical and Applied Genetics 80: 488-496.

Melchinger, A.E., Kuntze, L., Gumber, R.K., Lubberstedt, T., Fuchs, E. (1998). Genetic basis of resistance to sugarcane mosaic virus in European maize germplasm. Theoretical and Applied Genetics 96: 1151-1161.

Melchinger, A.E.: Genetic diversity and heterosis. In: Coors, J.G., Pandey, S. (eds) The genetics and exploitation of heterosis in corps, pp. 99-118. ASA-CSSA-SSSA, Madison (1999).

Meng, E., Ekboir, J.: Current and future trends in maize production and trade. In: Pingali, P.L. (ed) CIMMYT 1999-2000 world maize facts and trends. Meeting world maize needs: technological opportunities and priorities for the public sector, pp. 35-44, Mexico (2001).

Meyer, R.C., Törjék, O., Becher, M., Altmann, T. (2004). Heterosis of biomass production in *Arabidopsis*. Establishment during early development. Plant Physiology 134: 1813-1823.

Mikel, M.A., Cleo´ra, J.D.A., Rhodes, A.M. (1984). Genetics of resistance of two dent corn inbreds to maize dwarf mosiac virus and transfer of resistance into sweet corn. Phytopathology 74: 467-473.

Milach, S.C.K., Rines, H.W., Phillips, R.L. (2002). Plant height components and gibberellic acid response of oat dwarf lines. Crop Science 42: 1147-1154.

Mochizuki, A., Nishizawa, Y., Onodera, H., Tabei, Y., Toki, S., Habu, Y., Ugaki, M., Ohashi, Y. (1999). Transgenic rice plants expressing a trypsin inhibitor are resistant against rice stem borers, Chilo suppressalis. Entomologia Experimentalis Et Applicata 93: 173-178.

Moll, R.H., Lonnquist, J.H., Vélez Fortuno, J., Johnson, E.C. (1965). The relationship of heterosis and getetic divergence in maize. Genetics 52: 139-144.

Moore, G., Devos, K.M., Wang, Z., Gale, M.D. (1995). Cereal genome evolution: grasses, line up and form a circle. Current Biology 5: 737-739.

Mueller, E., Gilbert, J., Davenport, G., Brigneti, G., Baulcombe, D.C. (1995). Homology-dependent resistance - transgenic virus-resistance in plants related to homology-dependent gene silencing. Plant Journal 7: 1001-1013.

Müller, H.-J., Röder, T.: Microarrays. Elsevier, Amsterdam, Bostin, Heidelberg, London, New York, Oxford, Paris, San Diego, San Francisco, Singapore, Sydney, Tokyo (2006).

Nakazono, M., Qiu, F., Borsuk, L.A., Schnable, P.S. (2003). Laser-capture microdissection, a tool for the global analysis of gene expression in specific plant cell types: identification of genes expressed differentially in epidermal cells or vascular tissues of maize. Plant Cell 15: 583-596.

Niklas, K.J., Enquist, B.J. (2000). Invariant scaling relationships for interspecific plant biomass production rates and body size. Proceedings of the National Academy of Sciences of the United States of America 98: 2922-2927.

Oettler, G.: Improving falling number by recurrent selection in winter triticale. Proceedings of the 5th international triticale symposium, pp. 253-260, Radzików, Poland (2002).

Ogawa, M., Kusano, T., Koizumi, N., Katsumi, M., Sano, H. (1999). Gibberellin-responsive genes: high level of transcript accumulation in leaf sheath meristematic tissue from *Zea mays* L. Plant Molecular Biology 40: 645-657.

Olszewski, N., Sun, T., Gubler, F. (2002). Gibberellin signaling: biosynthesis, catabolism, and response pathways. Plant Cell Supplement 2002: S61-S80

Page, G.P., Ruden, D.M.: Combining high dimensional biological data to study complex diseases and quantitative traits. In: Allison, D.B., Page, G.P., Beasley, T.M., Edwards, J.W. (eds) DNA microarrays and related genomics techniques. Design, analysis and interpretation of experiments, pp. 335-360. Chapman & Hall/CRC, Boca, Raton, London, New York (2006).

Paliwal, R.L.: Introduction to maize and its importance. In: Paliwal, R.L., Granados, G., Lafitte, H.R., Violic, A.D. (eds) Tropical maize. Improvement and production, pp. 1-3. Food and Agricultural Organization of the United Nations, Rome (2000) a.

Paliwal, R.L.: Origin, evolution and spread of maize. In: Paliwal, R.L., Granados, G., Lafitte, H.R., Violic, A.D. (eds) Tropical maize. Improvement and production, pp. 5-11. Food and Agricultural Organization of the United Nations, Rome (2000) b.

Paliwal, R.L.: Maize types. In: Paliwal, R.L., Granados, G., Lafitte, H.R., Violic, A.D. (eds) Tropical maize. Improvement and production, pp. 39-43. Food and Agriculture Organization of the United Nations, Rome (2000) c.

Paliwal, R.L.: Uses of maize. In: Paliwal, R.L., Granados, G., Lafitte, H.R., Violic, A.D. (eds) Tropical maize. Improvement and production, pp. 45-57. Food and Agriculture Organization of the United Nations, Rome (2000) d.

Paliwal, R.L.: Hybrid maize breeding. In: Paliwal, R.L., Granados, G., Lafitte, H.R., Violic, A.D. (eds) Tropical maize. Improvement and production, pp. 143-160. Food and Agriculture Organization of the United Nations, Rome (2000) e.

Paterson, A.H., Lander, E.S., Hewitt, J.D., Peterson, S., Lincoln, S.E., Tanksley, S.D. (1988). Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335: 721-726.

Pedersen, K., Devereux, J., Wilson, D.R., Sheldon, E., Larkins, B.A. (1982). Cloning and sequence-analysis reveal structural variation among related zein genes in maize. Cell 29: 1015-1026.

Peterson, P.A., Bianchi, A.: Maize genetics and breeding in the 20th century. World Scientific, Singapore, New Jersey, London, Hong Kong (1999).

Pokorny, R., Porubova, M. (2006). Movement of Sugarcane mosaic virus in plants of resistant and susceptible maize lines. Cereal Research Communications 34: 1109-1116.

Quint, M., Mihaljevic, R., Dussle, C.M., Xu, M.L., Melchinger, A.E., Lubberstedt, T. (2002). Development of RGA-CAPS markers and genetic mapping of candidate genes for sugarcane mosaic virus resistance in maize. Theoretical and Applied Genetics 105: 355-363.

Raghavachari, N., Bao, Y.P., Li, G.S., Xie, X.Y., Muller, U.R. (2003). Reduction of autofluorescence on DNA microarrays and slide surfaces by treatment with sodium borohydride. Analytical Biochemistry 312: 101-105.

Rajeevan, M.S., Vernon, S.D., Taysavang, N., Unger, E.R. (2001). Validation of array-based gene expression profiles by real-time (kinetic) RT-PCR. Journal of Molecular Diagnostics 3: 26-31.

Randle, W., Davis, D.W., Groth, J.V. (1984). Improvement and genetic control of partial resistance in sweet corn to corn leaf rust. Journal of American Society Of Horticulture Science 109: 777-781.

Redinbaugh, M.G., Jones, M.W., Gingery, R.E. (2004). The genetics of virus resistance in maize (*Zea mays* L.). Maydica 49: 183-190.

Redman, J.C., Haas, B.J., Tanimoto, G., Town, C.D. (2004). Development and evaluation of an *Arabidopsis* whole genome Affymetrix probe array. Plant Journal 38: 545-561.

Rensink, W.A., Buell, C.R. (2005). Microarray expression profiling resources for plant genomics. Trends in Plant Science 10: 603-609.

Reymond, P., Weber, H., Damond, M., Farmer, E.E. (2000). Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. Plant Cell 12: 707-719.

Rhodes, C.A., Pierce, D.A., Mettler, I.J., Mascarenhas, D., Detmer, J.J. (1988). Genetically transformed maize plants from protoplasts. Science 240: 204-207.

Ribaut, J.M., Betrán, J. (1999). Single large-scale marker-assisted selection (SLS-MAS). Molecular Breeding 5: 531-541.

Ribaut, J.M., Banziger, M., Betrán, J., Jinang, C., Edmeades, G.O., Dreher, K., Hosington, D.: Use of molecular markers in plant breeding: drought tolerance improvement in tropical maize. In: Kang, M.S. (ed) Quantitative Genetics, Genomics and Plant Breeding, pp. 85-99. CABI International, Wallingford (2002).

Richey, F.D. (1922). The experimental basis for the present status of corn breeding. Journal of the American Society of Agronomy 14: 1-17.

Roane, C.W., Genter, C.F., Tolin, S.A. (1977). Inheritance of resistance to maize dwarf mosaic virus in maize (Abstr.). Proceedings of the American Phytopathology Society 4: 140.

Roda, A.L., Baldwin, I.T. (2003). Molecular technology reveals how the induced direct defenses of plants work. Basic and Applied Ecology 4: 15-26.

Roelfs, A.P. (1988). Genetic-control of phenotypes in wheat-stem rust. Annual Review of Phytopathology 26: 351-367.

Romagnoli, S., Maddaloni, M., Livini, C., Motto, M. (1990). Relationship between gene-expression and hybrid vigor in primary root-tips of young maize (*Zea mays* L.) plantlets. Theoretical and Applied Genetics 80: 769-775.

Rood, S.B., Blake, T.J., Pharis, R.P. (1983) a. Gibberellins and heterosis in maize. 2. Response to gibberellic-acid and metabolism of [H-3]-labeled gibberellin-a20. Plant Physiology 71: 645-651.

Rood, S.B., Pharis, R.P., Koshioka, M., Major, D.J. (1983) b. Gibberellins and heterosis in maize. 1. Endogenous gibberellin like substances. Plant Physiology 71: 639-644.

Ros, B., Thümmler, F., Wenzel, G. (2004). Analysis of differentially expressed genes in a susceptable and moderately resistant potato cultivar upon *Phytophthora infestans* infection. Molecular Plant Pathology 5: 191-201.

Rosenkranz, E., Scott, G.E. (1984). Determination of the number of genes for resistance to maize dwarf mosaic virus strain A in five corn inbred lines. Phytopathology 74: 71-76.

Ruan, Y., Gilmore, J., Conner, T. (1998). Towards *Arabidopsis* genome analysis: monitoring expression profiles of 1400 genes using cDNA microarrays. Plant Journal 15: 821-833.

Rubin, M.A. (2001). Use of laser capture microdissection, cDNA microarrays, and tissue microarrays in advancing our understanding of prostate cancer. Journal of Pathology 195: 80-86.

Sari-Gorla, M., Krajewski, P., Fonzo, N.D., Villa, M., Frova, C. (1999). Genetic analysis of drought tolerance in maize by molecular markers. II. Plant height and flowering. Theoretical and Applied Genetics 99: 289-295.

Sarkissian, I.V., Srivastava, H.K. (1969). High efficiency, heterosis and homeostasis in mitochondria of wheat. Proceedings of the National Academy of Sciences of the United States of America 63: 302-309.

Schadt, E.E., Monks, S.A., Drake, T.A., Lusis, A.J., Che, N., Colinayo, V., Ruff, T.G., Milligan, S.B., Lamb, J.R., Cavet, G., Linsley, P.S., Mao, M., Stoughton, R.B., Friend, S.H. (2003). Genetics of gene expression surveyed in maize, mouse and man. Nature 422: 297-302.

Scheideler, M., Schlaich, N.L., Fellenberg, K., Beissbarth, T., Hauser, N.C., Vingron, M., Slusarenko, A.J., Hoheisel, J.D. (2002). Monitoring the switch from housekeeping to pathogen defense metabolism in *Arabidopsis thaliana* using cDNA arrays. Journal of Biological Chemistry 277: 10555-10561.

Schena, M., Davis, R.W.: Genes, genomes, and chips. In: Schena, M. (ed) DNA microarrays. A practical approach, pp. 1-16. University Press, Oxford (1999).

Schena, M.: Microarray analysis. Wiley-Liss, Hoboken, New Jersey (2003).

Schmittgen, T.D., Zakrajsek, B.A. (2000). Effect of experimental treatment on housekeeping gene expression: validation by real-time, quantitative RT-PCR. Journal of Biochemical and Biophysical Methods 46: 69-81.

Schmittgen, T.D., Zakrajsek, B.A., Mills, A.G., Gorn, V., Singer, M.J., Reed, M.W. (2000). Quantitative reverse transcription-polymerase chain reaction to study mRNA decay: Comparison of endpoint and real-time methods. Analytical Biochemistry 285: 194-204.

Schnable, P.S., Hochholdinger, F., Nakazano, M. (2004). Global expression profiling applied to plant development. Current Opinion in Plant Biology 7: 50-56.

Schneider, K.A., Brothers, M.E., Kelly, J.D. (1997). Marker-assisted selection to improve drought resistance in common bean. Crop Science 37: 51-60.

Sehgal, S.M.: Effects of teosinte and *Tripsacum* introgression in maize. University Cambridge, Harvard (1963).

Seki, M., Narusaka, M., Abe, H., Kasuga, M., Yamaguchi-Shinozaki, K., Carninci, P., Hayashizaki, Y., Shinozaki, K. (2001). Monitoring the expression pattern of 1300 *Arabidopsis* genes under drought and cold stresses by using a full-length cDNA microarray. Plant Cell 13: 61-72.

Shi, C., Ingvardsen, C., Thummler, F., Melchinger, A.E., Wenzel, G., Lubberstedt, T. (2005). Identification by suppression subtractive hybridization of genes that are differentially expressed between near-isogenic maize lines in association with sugarcane mosaic virus resistance. Molecular Genetics and Genomics 273: 450-461.

Shi, C., Uzarowska, A., Ouzunova, M., Landbeck, M., Wenzel, G., Lubberstedt, T. (2007). Identification of candidate genes associated with cell wall digestibility and eQTL (expression quantitative trait loci) analysis in a flint x flint maize recombinant inbred line population. BMC Genomics 8: 22.

Shi, L.M., Tong, W.D., Su, Z.Q., Han, T., Han, J., Puri, R.K., Fang, H., Frueh, F.W., Goodsaid, F.M., Guo, L., Branham, W.S., Chen, J.J., Xu, Z.A., Harris, S.C., Hong, H.X., Xie, Q., Perkins, R.G., Fuscoe, J.C. (2005). Microarray scanner calibration curves: characteristics and implications. BMC Bioinformatics 6 (Suppl 2): S11.

Shukla, D.D., Frenkel, M.J., Mckern, N.M., Ward, C.W., Jilka, J., Tosic, M., Ford, R.E. (1992) a. Present status of the sugarcane mosaic subgroup of potyviruses. Archives of Virology: 363-373.

Shukla, D.D., Lauricella, R., Ward, C.W. (1992) b. Serology of potyviruses - current problems and some solutions. Archives of Virology: 57-69.

Shull, G.H. (1908). The composition of a field of maize. American Breeders Association Reports 4: 296-301.

Shull, G.H. (1909). A pure-line method of corn breeding. American Breeders Association Reports 5: 51-59.

Slonim, D.K. (2002). From patterns to pathways: gene expression data analysis comes of age. Nature Genetics 32: 502-508.

Smith, D.R., White, D.G.: Diseases of corn. In: Spraque, G.F., Dudley, J.W. (eds) Corn and corn improvement, 3rd ed, pp. 687-766. ASA-CSSA-SSSA, Madison (1988).

Smith, O.S., Smith, J.C.S., Bowen, S.L., Tenborg, R.A., Wall, S.J. (1990). Similarities among a group of elite maize inbreds as measured by pedigree, F1 grain yield, grain yield, heterosis, and RFLPs. Theoretical and Applied Genetics 80: 833-840.

Sowiński, P., Rudzinska-Langwald, A., Adamczyk, J., Kubica, W., Fronk, J. (2005). Recovery of maize seedling growth, development and photosynthetic efficiency after initial growth at low temperature. Journal of Plant Physiology 162: 67-80.

Sprague, G.F., Dudley, J.W.: Corn and corn improvement: 3rd edition. Cambridge University Press, Madison (1988).

Steckel, D.: Microarray Bioinformatics. Cambridge University Press, New York (2003).

Stokes, D., Morgan, C., O'Neill, C., Fraser, F., Bancroft, I.: Transcriptome-based predictive modelling of heterosis. Heterosis in plants, Potsdam-Golm, Germany (2006).

Stuber, C.W., Edwards, M.D., Wendel, J.F. (1987). Molecular marker-facilitated investigations of quantitative trait loci in maize. 2. Factors influencing yield and its component traits. Crop Science 27: 639-648.

Stuber, C.W., Lincoln, S.E., Wolff, D.W., Helentjaris, T., Lander, E.S. (1992). Identification of genetic-factors contributing to heterosis in a hybrid from 2 elite maize inbred lines using molecular markers. Genetics 132: 823-839.

Stuber, C.W.: Breeding multigenie traits. In: Phillips, R., Vasil, I. (eds) DNA-based markers in plants, pp. 97-115. Kluwer Academic Publishers, Dordrecht (1994) a.

Stuber, C.W.: Heterosis in plant breeding. In: Janick, J. (ed) Plant Breeding Reviews, pp. 227-251. John Wiley & Sons, New York (1994) b.

Swanson-Wagner, R.A., Jia, Y., DeCook, R., Borsuk, L.A., Nettleton, D., Schnable, P.S. (2006). All possible modes of gene action are observed in a global comparison of gene expression in a maize F1 hybrid and its inbred parents. Proceedings of the National Academy of Sciences of the United States of America 103: 6805-6810.

Tanaka, R., Oster, U., Kruse, E., Rüdiger, W., Grimm, B. (1999). Reduced activity of geranylgeranyl reductase leads to loss of chlorophyll and tocopherol and to partially geranylgeranylated chlorophyll in transgenic tobacco plants expressing antisense RNA for geranylgeranyl reductase. Plant Physiology 120: 695-704.

Tanksley, S.D. (1993). Mapping polygenes. Annual Review of Genetics 27: 205-233.

Taylor, S., Massiah, A., Lomonossoff, G., Roberts, L.M., Lord, J.M., Hartley, M. (1994). Correlation between the activities of 5 ribosome-inactivating proteins in depurination of tobacco ribosomes and inhibition of tobacco mosaic-virus infection. Plant Journal 5: 827-835.

Thellin, O., Zorzi, W., Lakaye, B., De Borman, B., Coumans, B., Hennen, G., Grisar, T., Igout, A., Heinen, E. (1999). Housekeeping genes as internal standards: use and limits. Journal of Biotechnology 75: 291-295.

Toenniessen, G.H., O'Toole, J.C., DeVries, J. (2003). Advances in plant biotechnology and its adoption in developing countries. Current Opinion in Plant Biology 6: 191-198.

Tollenaar, M., Lee, E.A. (2002). Yield potential, yield stability and stress tolerance in maize. Field Crop Research 75: 161-169.

Trethewey, R.N. (2001). Gene discovery via metabolic profiling. Current Opinion in Biotechnology 12: 135-138.

Troyer, A.F., Larkins, J.R. (1985). Selection for early flowering in corn: 10 late synthetics. Crop Science 25: 695-697.

Troyer, A.F. (2006). Adaptedness and heterosis in corn and mule hybrids. Crop Science 46: 528-543.

Torp, J., Jørgensen, J.H. (1986). Modification of barley powdery mildew resistance gene *Ml-a12* by induced mutation. Canadian Journal of Genetics and Cytology 28: 725-731.

Tsaftaris, S.A. (1995). Molecular aspects of heterosis in plants. Physiologia Plantarum 94: 362-370.

Tseng, G.C., Oh, M.K., Rohlin, L., Liao, J.C., Wong, W.H. (2001). Issues in cDNA microarray analysis: quality filtering, channel normalization, models of variations and assessment of gene effects. Nucleic Acids Research 29: 2549-2557.

Tu, J.C., Ford, R.E. (1969) a. Infectivity changes of maize dwarf mosaic virus *in-vivo* and *in-vitro*. Phytopathology 59: 1947.

Tu, J.C., Ford, R.E. (1969) b. Interaction of maize dwarf mosaic virus strains and sugarcane mosaic virus H in corn. Phytopathology 59: 173-178.

Tuinstra, M.R., Ejeta, G., Goldsbrough, P.B. (1997). Heterogeneous inbred family (HIF) analysis: a method for developing near-isogenic lines that differ at quantitative trait loci. Theoretical and Applied Genetics 95: 1005-1011.

Uzarowska, A., Keller, B., Piepho, H.P., Schwarz, G., Ingvardsen, C., Wenzel, G., Lubberstedt, T. (2007). Comparative expression profiling in meristems of inbred-hybrid triplets of maize based on morphological investigations of heterosis for plant height. Plant Molecular Biology 63: 21-34.

Van Koevering, M., Haufler, Z.K., Fulbright, D.W., Isleib, T.G., Everson, E.H. (1987). Heritability of resistance in winter wheat to wheat spindle streak mosaic virus. Phytopathology 77: 742-744.

Vodkin, L.O., Khanna, A., Shealy, R., Clough, S.J., Gonzalez, D.O., Philip, R., Zabala, G., Thibaud-Nissen, F., Sidarous, M., Stromvik, M.V., Shoop, E., Schmidt, C., Retzel, E., Erpelding, J., Shoemaker, R.C., Rodriguez-Huete, A.M., Polacco, J.C., Coryell, V., Keim, P., Gong, G., Liu, L., Pardinas, J., Schweitzer, P. (2004). Microarrays for global expression constructed with a low redundancy set of 27,500 sequenced cDNAs representing an array of developmental stages and physiological conditions of the soybean plant. Bmc Genomics 5: -.

Waterhouse, P.M., Graham, H.W., Wang, M.B. (1998). Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. Proceedings of the National Academy of Sciences of the United States of America 95: 13959-13964.

Weber, D.F.: The production and utilization of monosomic *Zea mays* in cytogenetic studies. In: Reddy, G.M., Coe, E.H. (eds) Gene structure and function in higher plants, pp. 191-204. Oxford and IBH Publishing Company, New Delhi (1986).

Wendel, J.F., Stuber, C.W., Edwards, M.D., Goodman, M.M. (1986). Duplicated chromosome segments in maize (*Zea mays* L.): further evidence from hexokinase isozymes. Theoretical and Applied Genetetics 72: 178-185.

Wesley, S.V., Helliwell, C.A., Smith, N.A., Wang, M.B., Rouse, D.T., Liu, Q., Gooding, P.S., Singh, S.P., Abbott, D., Stoutjesdijk, P.A., Robinson, S.P., Gleave, A.P., Green, A.G., Waterhouse, P.M. (2001). Construct design for efficient, effective and high-throughput gene silencing in plants. Plant Journal 27: 581-590.

Whitham, S.A., Quan, S., Hur-Song, C., Cooper, B., Estes, B., Tong, Z., Wang, X., Yu-Ming, H. (2003). Diverse RNA viruses elicit the expression of common sets of genes in susceptable *Arabidopsis thaliana* plants. Plant Journal 33: 271-283.

Williams, W. (1959). Heterosis and the genetics of complex characters. Nature (London) 184: 527-530.

Wisman, E., Ohlrogge, J. (2000). Arabidopsis microarray service facilities. Plant Physiology 124: 1468-1471.

Xia, X.C., Melchinger, A.E., Kuntze, L., Lubberstedt, T. (1999). Quantitative trait loci mapping of resistance to sugarcane mosaic virus in maize. Phytopathology 89: 660-667.

Xiao, J., Li, J., Yuan, L., McCouch, S.R., Tanksley, S.D. (1996). Genetic diversity and its relationship to hybrid performance and heterosis in rice as revealed by PCR-based markers. Theoretical and Applied Genetics 92: 637-643.

Xiao, J.H., Li, J.M., Yuan, L.P., Tanksley, S.D. (1995). Dominance is the major genetic basis of heterosis in rice as revealed by QTL analysis using molecular markers. Genetics 140: 745-754.

Xing, Y., Ingvardsen, C., Salomon, R., Lübberstedt, T. (2006). Analysis of sugarcane mosaic virus resistance in maize in an isogenic dihybrid crossing scheme and implications for breeding potyvirus-resistant maize hybrids. Genome 49: 1274-1282.

Xu, M.L., Melchinger, A.E., Xia, X.C., Lubberstedt, T. (1999). High-resolution mapping of loci conferring resistance to sugarcane mosaic virus in maize using RFLP, SSR, and AFLP markers. Molecular and General Genetics 261: 574-581.

Yamauchi, Y., Ogawa, M., Kuwahara, A., Hanada, A., Kamiya, Y., Yamaguchi, S. (2004). Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of *Arabidopsis thaliana* seeds. Plant Cell 16: 367-378.

Yang, Y.H., Dudoit, S., Luu, P., Lin, D.M., Peng, V., Ngai, J., Speed, T.P. (2002). Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. Nucleic Acids Research 30: e15.

Yang, Y.H., Speed, T. (2002). Design issues for cDNA microarray experiments. Nature Reviews Genetics 3: 579-588.

Yousef, G.G., Juvik, J.A. (2001). Comparison of phenotypic and marker-assisted selection for quantitative traits in sweet corn. Crop Science 41: 645-655.

Yu, S.B., Li, J.X., Tan, Y.F., Gao, Y.J., Li, X.H., Zhang, Q.F., Maroof, M.A.S. (1997). Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. Proceedings of the National Academy of Sciences of the United States of America 94: 9226-9231.

Yuan, L., Dussle, C.M., Melchinger, A.E., Utz, H.F., Lubberstedt, T. (2003). Clustering of QTL conferring SCMV resistance in maize. Maydica 48: 55-62.

Yuen, T., Wurmbach, E., Pfeffer, R.L., Ebersole, B.J., Sealfon, S.C. (2002). Accuracy and calibration of commercial oligonucleotide and custom cDNA microarrays. Nucleic Acids Research 30: e48.

Zhang, S.H., Li, X.H., Wang, Z.H., George, M.L., Jeffers, D., Wang, F.G., Liu, X.D., Li, M.S., Yuan, L.X. (2003). QTL mapping for resistance to SCMV in Chinese maize germplasm. Maydica 48: 307-312.

Zhong, Y.W., Guo, A.Y., Li, C.B., Zhuang, B.Q., Lai, M., Wei, C.H., Luo, J.C., Li, Y. (2005). Identification of a naturally occurring recombinant isolate of sugarcane mosaic virus causing maize dwarf mosaic disease. Virus Genes 30: 75-83.

Zinselmeier, C., Sun, Y.J., Helentjaris, T., Beatty, M., Yang, S., Smith, H., Habben, J. (2002). The use of gene expression profiling to dissect the stress sensitivity of reproductive development in maize. Field Crops Research 75: 111-121.

Zsubori, Z., Gyenes-Hegyi, Z., Illés, O., Pók, I., Rácz, F., Szőke, C. (2002). Inheritance of plant and ear height in maize (*Zea mays* L.). Journal of Agricultural Sciences, University of Debrecen 8: 34-38.

8 ANNEX

Table 1. Genotype 002x301 vs. 301, significantly differentially expressed genes (without interaction)

EST	Estimate	StdErr	tValue	Raw p	Upregulated	FDR p ≤ 0.05
MEST289-B04	-3.91773	0.5776	-6.78278	4.84E-05	301	0.008601
MEST114-C10	-1.60215	0.370951	-4.31904	0.000235	301	0.027504
MEST379-A05	-1.51797	0.351508	-4.31845	0.00037	301	0.036429
618026B08.x1	-1.1572	0.285777	-4.04933	0.000465	301	0.043174
MEST73-G09	-0.91782	0.223193	-4.11225	0.000311	301	0.032485
687063G07.y1	-0.61748	0.14816	-4.16766	0.000146	301	0.019449
MEST36-A10	-0.5244	0.108375	-4.83874	1.71E-05	301	0.004322
707082E08.x1	-0.43974	0.091785	-4.79102	1.7E-05	301	0.004322
MEST101-C01	-0.40224	0.087511	-4.59641	3.24E-05	301	0.006381
MEST350-H05	-0.3963	0.101556	-3.90228	0.00033	301	0.033936
MEST31-H08	-0.37497	0.092216	-4.06622	0.000181	301	0.022799
486075E05.x1	-0.32499	0.071941	-4.51741	4.5E-05	301	0.008156
486066D07.x1	-0.30154	0.058559	-5.14938	4.84E-06	301	0.001754
606040H08.x1	-0.2611	0.061512	-4.24481	9.96E-05	301	0.015404
614091D05.x1	-0.248	0.06019	-4.12023	0.000156	301	0.02035
618013C05.x1	-0.23478	0.047021	-4.99313	8.25E-06	301	0.002455
MEST36-G07	-0.23432	0.03833	-6.11331	1.81E-07	301	0.000124
614091C05.x1	-0.22517	0.053934	-4.1749	0.000128	301	0.017668
707056A08.x2	-0.14343	0.035883	-3.99717	0.00022	301	0.026573
614078C08.x1	0.139026	0.033101	4.200063	0.000115	002x301	0.016481
486089A05.x1	0.156182	0.04013	3.891918	0.000306	002x301	0.032485
486018D09.x4	0.157237	0.031266	5.028941	7.3E-06	002x301	0.00223
614001F02.x6	0.1786	0.04295	4.158353	0.000135	002x301	0.018323
606021A06.x2	0.196481	0.039448	4.980773	8.61E-06	002x301	0.002497
707082G05.x1	0.198647	0.053046	3.744794	0.000484	002x301	0.044174
606013A11.x2	0.202829	0.051888	3.908975	0.000303	002x301	0.032485
MEST106-E11	0.21896	0.056318	3.887894	0.000316	002x301	0.032781
MEST22-C06	0.223529	0.059458	3.759479	0.00047	002x301	0.043326
707061C08.x1	0.224622	0.056203	3.996649	0.00022	002x301	0.026573
486091F11.x1	0.227259	0.050875	4.467032	4.97E-05	002x301	0.008601
707050A02.y1	0.228169	0.058281	3.914955	0.000291	002x301	0.032449
614044B10.x4	0.241491	0.059117	4.084978	0.000166	002x301	0.021456
707020B03.y1	0.245031	0.047733	5.133387	5.11E-06	002x301	0.001764
603005C02.x1	0.250621	0.042248	5.932109	3.18E-07	002x301	0.000185
496022B11.x1	0.25497	0.060456	4.217487	0.000115	002x301	0.016481
MEST24-C11	0.263682	0.050385	5.233356	3.63E-06	002x301	0.001451
MEST40-H02	0.276823	0.066728	4.148514	0.000136	002x301	0.018323
614083E04.y1	0.278457	0.0445	6.257407	1.01E-07	002x301	7.34E-05
MEST14-G03	0.280534	0.06534	4.293475	8.98E-05	002x301	0.014086
707057A10.x1	0.290017	0.064075	4.52619	4.09E-05	002x301	0.007723

EST	Estimate	StdErr	tValue	Raw p	Upregulated	FDR p ≤ 0.05
603030C03.x1	0.29118	0.064371	4.523463	4.13E-05	002x301	0.007723
707030B09.x2	0.297568	0.060695	4.902681	1.12E-05	002x301	0.0031
614018B03.x1	0.300377	0.063483	4.731609	2.07E-05	002x301	0.004807
486024H12.x1	0.304317	0.065481	4.647414	2.84E-05	002x301	0.005884
496024C07.x1	0.309822	0.077509	3.997221	0.000225	002x301	0.026612
MEST257-B03	0.326078	0.085586	3.809929	0.000411	002x301	0.039065
707064F09.y1	0.330794	0.080931	4.087334	0.000169	002x301	0.021557
606029C04.x1	0.332293	0.084438	3.935336	0.000299	002x301	0.032485
618046D03.y1	0.341539	0.067031	5.095216	5.82E-06	002x301	0.001905
606025A12.x1	0.357438	0.091418	3.909941	0.000308	002x301	0.032485
MEST35-B01	0.360692	0.097281	3.707735	0.000551	002x301	0.048446
486058B05.x2	0.361098	0.086247	4.186809	0.000126	002x301	0.017664
MEST11-B05	0.368468	0.097752	3.769407	0.000556	002x301	0.048516
614020G04.y1	0.390677	0.080359	4.861673	1.46E-05	002x301	0.003929
614045E12.x4	0.426759	0.077778	5.486873	1.51E-06	002x301	0.000729
MEST18-C05	0.440367	0.093505	4.709565	2.6E-05	002x301	0.005703
606054C07.x2	0.442108	0.081126	5.449617	2.16E-06	002x301	0.001001
MEST500-B02	0.44589	0.093231	4.782631	1.97E-05	002x301	0.004762
496030G01.x1	0.466853	0.115937	4.026769	0.000215	002x301	0.02649
MEST13-G06	0.499367	0.103308	4.833767	1.6E-05	002x301	0.00421
MEST19-E10	0.518525	0.135497	3.826849	0.000435	002x301	0.040733
MEST253-B10	0.588488	0.134689	4.369247	8.61E-05	002x301	0.013871
618028A09.x1	0.609188	0.071384	8.534004	4.89E-11	002x301	1.13E-07
618004F11.x1	0.617366	0.075884	8.135602	3.05E-10	002x301	5.05E-07
MEST12-C02	0.635662	0.14931	4.257332	0.000126	002x301	0.017664
614067C02.x1	0.658632	0.166835	3.947818	0.000281	002x301	0.032252
606055H07.x2	0.703841	0.160707	4.379641	0.000108	002x301	0.016213
707066F01.y1	0.705351	0.104038	6.779752	2.16E-08	002x301	2.28E-05
707064C07.y1	0.708812	0.163585	4.332979	8.97E-05	002x301	0.014086
486036C10.x1	0.790317	0.122242	6.465181	9.43E-08	002x301	7.34E-05
687062G06.x1	0.818365	0.142889	5.72728	1.06E-06	002x301	0.000533
MEST19-G09	0.830184	0.091618	9.061315	1.28E-11	002x301	4.93E-08
MEST20-H012	0.863412	0.182084	4.741825	2.56E-05	002x301	0.005703
618046F07.y1	0.89582	0.233024	3.844331	0.000522	002x301	0.046384
687066C08.x1	0.951032	0.233681	4.069792	0.000222	002x301	0.026573
707041F03.x1	1.191535	0.255389	4.665574	3.75E-05	002x301	0.007256
MEST43-B01	1.319334	0.20245	6.516852	9.94E-08	002x301	7.34E-05
614074F08.y3	1.752565	0.26302	6.66324	8.49E-07	002x301	0.000448
614020G04.y1	1.838692	0.26029	7.064008	4.38E-08	002x301	3.91E-05
606016G10.x2	1.887019	0.434771	4.340256	0.000288	002x301	0.032449

Table 2. Genotype 005x250 vs. 005, significantly differentially expressed genes (without interaction)

EST	Estimate	StdErr	tValue	Raw p	Upregulated	FDR p ≤ 0.05
614070D09.x1	-0.16821	0.041218	-4.0809	0.000177	005	0.026681
614041D11.x2	-2.95416	0.615843	-4.79693	0.000349	005	0.044248
614074A05.x3	-0.21785	0.051039	-4.26826	9.74E-05	005	0.018151
945002E10.X3	-0.12886	0.029497	-4.3687	6.65E-05	005	0.013448
606032C10.x1	-0.12758	0.032874	-3.88082	0.000317	005	0.041109
683003D11.x1	-0.16254	0.039035	-4.16398	0.000129	005	0.022336
MEST27-A10	-0.18234	0.039154	-4.65695	2.66E-05	005	0.007724
707056E08.x1	-0.17999	0.045269	-3.97592	0.00024	005	0.033719
486051H06.x2	-1.75412	0.381702	-4.59553	0.000224	005	0.032643
486024F06.x1	-0.85149	0.200625	-4.24418	0.000141	005	0.023072
486036C10.x1	-0.42308	0.10955	-3.86195	0.000392	005	0.046742
MEST137-B02	-0.18274	0.042892	-4.26032	9.72E-05	005	0.018151
MEST42-B03	-0.60275	0.153447	-3.9281	0.000313	005	0.041109
MEST15-E12	-0.36164	0.076434	-4.73143	2.91E-05	005	0.007892
707090D10.x1	-0.19501	0.047052	-4.14459	0.000138	005	0.02278
606011D07.x1	-0.27921	0.070284	-3.97258	0.000316	005	0.041109
606036F05.x1	-0.69194	0.179151	-3.86234	0.000382	005	0.046021
687042F04.x2	-1.53249	0.318328	-4.81417	0.000338	005	0.043362
707017F05.x1	0.913398	0.16195	5.639997	1.14E-06	005x250	0.000905
707066F01.y1	0.53248	0.099266	5.364197	2.71E-06	005x250	0.001629
605002G07.x1	0.153924	0.040598	3.791412	0.000419	005x250	0.04896
606055H07.x2	0.760643	0.17507	4.344785	0.000119	005x250	0.020923
486068C07.x1	0.295907	0.067506	4.383421	6.34E-05	005x250	0.013066
MEST22-F02	0.391487	0.101329	3.863529	0.000356	005x250	0.044649
MEST348-D12	0.886179	0.209717	4.225585	0.000134	005x250	0.02278
614084F03.x1	0.202781	0.041478	4.888928	1.18E-05	005x250	0.004835
614069C07.y1	0.675735	0.134382	5.028462	1.08E-05	005x250	0.004606
606008C10.x1	0.291423	0.04772	6.106875	2E-07	005x250	0.000265
606025F01.x1	0.122729	0.03246	3.780972	0.00044	005x250	0.050997
606066C12.y1	0.228843	0.049028	4.667572	2.48E-05	005x250	0.007724
606058B03.x2	0.416439	0.063665	6.541134	3.72E-08	005x250	7.39E-05
486040E09.x1	0.443721	0.113099	3.923283	0.000289	005x250	0.038787
486020B09.x2	0.442098	0.079975	5.527965	1.56E-06	005x250	0.001162
486069G09.x1	0.226116	0.059039	3.829937	0.000379	005x250	0.046021
687062G06.x1	1.040953	0.139112	7.482845	3.46E-09	005x250	1.03E-05
683003H10.x1	0.850601	0.216238	3.933641	0.000287	005x250	0.038787
MEST15-H09	0.158168	0.039705	3.983561	0.000235	005x250	0.033713
MEST8-A01	0.600853	0.118029	5.090719	7.14E-06	005x250	0.003407
MEST43-B12	0.200269	0.035722	5.606362	1.06E-06	005x250	0.000899
MEST77-F03	1.116241	0.2323	4.805163	3.5E-05	005x250	0.008876
MEST55-H08	1.619481	0.3136	5.164155	1.77E-05	005x250	0.006576
MEST31-C07	0.614505	0.130466	4.71007	3.84E-05	005x250	0.009539
MEST31-H08	0.430659	0.092205	4.670658	2.54E-05	005x250	0.007724

EST	Estimate	StdErr	tValue	Raw p	Upregulated	FDR p ≤ 0.05
MEST177-D11	0.407176	0.091768	4.436995	6.03E-05	005x250	0.012846
MEST12-C02	0.79806	0.146231	5.45753	2.93E-06	005x250	0.001629
606014D09.x1	0.216602	0.037493	5.777213	5.48E-07	005x250	0.000545
606021A06.x2	0.194703	0.039448	4.935692	1E-05	005x250	0.004449
606028A01.x1	0.217325	0.044748	4.856608	1.31E-05	005x250	0.005057
603031A08.x1	0.178353	0.033636	5.302449	3E-06	005x250	0.001629
603041A04.x1	0.339123	0.088367	3.837656	0.000363	005x250	0.045047
486092H04.y1	0.21716	0.038281	5.672836	7.89E-07	005x250	0.000724
486068D09.x1	0.23856	0.055452	4.302089	8.27E-05	005x250	0.01609
MEST42-E04	0.748669	0.160523	4.663946	3.28E-05	005x250	0.008511
MEST35-D12	1.509189	0.279032	5.408665	2.3E-05	005x250	0.007615
614010D03.x2	0.375795	0.081051	4.636547	2.84E-05	005x250	0.007892
614010A04.x2	0.401277	0.099312	4.040574	0.000201	005x250	0.029913
486093D03.y1	0.191179	0.04309	4.436701	5.32E-05	005x250	0.011978
486068B10.y1	0.187607	0.038635	4.855946	1.31E-05	005x250	0.005057
496022G05.x1	0.775644	0.174768	4.43813	5.81E-05	005x250	0.012795
MEST252-G01	0.763539	0.173099	4.411009	7.56E-05	005x250	0.015032
MEST384-G08	1.69733	0.357651	4.745768	0.00026	005x250	0.035931
614066F07.y1	1.238203	0.290816	4.257684	0.000154	005x250	0.024457
614032E07.y1	1.125155	0.264169	4.259217	0.000168	005x250	0.025715
614065H08.x1	0.198169	0.036809	5.383644	2.27E-06	005x250	0.001596
MEST67-G12	0.353615	0.083807	4.219384	0.000111	005x250	0.020041

Table 3. Genotype 005x250 vs. 250, significantly differentially expressed genes (without interaction)

EST	Estimate	StdErr	tValue	Raw p	Upregulated	FDR p ≤ 0.05
614072G07.x1	-2.06378	0.377085	-5.47299	5.11E-05	250	0.017889
MEST20-H09	-1.77121	0.395911	-4.47376	0.00026	250	0.04655
614053D11.x1	-1.59869	0.39413	-4.05626	0.000312	250	0.050253
MEST46-A07	-1.32578	0.293994	-4.50955	0.000174	250	0.036109
614035C03.x1	-1.25724	0.247581	-5.07811	2.47E-05	250	0.010888
MEST12-C11	-1.25537	0.257503	-4.87518	2.34E-05	250	0.010705
614079H04.x1	-1.23605	0.251651	-4.91174	8.42E-05	250	0.02403
614064C10.y1	-1.15192	0.224087	-5.14052	3.29E-05	250	0.013073
MEST21-D04	-0.68899	0.163567	-4.21226	0.000131	250	0.031103
945031A04.Y1	-0.51407	0.1294	-3.97271	0.000248	250	0.046409
945031B02.X1	-0.33323	0.068802	-4.84332	1.43E-05	250	0.007717
486085H07.x2	-0.2292	0.053081	-4.31799	8.07E-05	250	0.02402
486073F06.x2	-0.22478	0.055092	-4.08018	0.000173	250	0.036109
618013C05.x1	-0.20061	0.047021	-4.26639	9.28E-05	250	0.02403
606014D09.x1	-0.16033	0.037493	-4.27644	8.99E-05	250	0.02403
MEST13-A12	0.123748	0.029989	4.126431	0.000146	005x250	0.032551
MEST27-A10	0.153394	0.039149	3.918241	0.000288	005x250	0.047603
MEST29-D11	0.156352	0.036841	4.243923	9.99E-05	005x250	0.025295
486028G05.x1	0.162786	0.021941	7.419197	1.68E-09	005x250	5E-06
MEST39-C05	0.1673	0.035772	4.676809	2.58E-05	005x250	0.01096
707090G04.x1	0.192833	0.042185	4.571097	3.53E-05	005x250	0.013159
707090D10.x1	0.195478	0.047052	4.154486	0.000133	005x250	0.031103
614074A05.x3	0.202002	0.051349	3.933926	0.00028	005x250	0.047603
MEST73-G12	0.211338	0.044449	4.754621	1.99E-05	005x250	0.009491
614070D09.x1	0.224031	0.041223	5.434554	2.02E-06	005x250	0.001602
MEST137-B02	0.26272	0.042878	6.127136	1.73E-07	005x250	0.000257
707017E10.x1	0.271808	0.059339	4.58062	3.54E-05	005x250	0.013159
496022B11.x1	0.29151	0.05927	4.918361	1.16E-05	005x250	0.006551
605018B04.x1	0.298636	0.043694	6.834647	1.32E-08	005x250	3.14E-05
MEST15-E12	0.339403	0.077617	4.372808	8.85E-05	005x250	0.02403
606020E08.x1	0.343405	0.06814	5.039692	8.06E-06	005x250	0.00505
486022E05.x2	0.352923	0.082363	4.284964	8.98E-05	005x250	0.02403
614028A08.y1	0.368123	0.081164	4.535563	3.97E-05	005x250	0.014308
606061B11.x1	0.390758	0.099132	3.941789	0.000262	005x250	0.04655
606029C04.x1	0.410983	0.084303	4.875085	1.52E-05	005x250	0.00788
603016C07.x1	0.431173	0.082655	5.216521	4.24E-06	005x250	0.002967
MEST31-D04	0.436569	0.111432	3.917795	0.000308	005x250	0.050213
MEST22-E10	0.44169	0.101313	4.359678	7.26E-05	005x250	0.022461
614084A02.x1	0.477443	0.119776	3.986122	0.000249	005x250	0.046409
486091C10.x1	0.481169	0.119189	4.037025	0.000194	005x250	0.038816
486021C06.x3	0.48251	0.11175	4.317768	9.11E-05	005x250	0.02403
486022B02.x2	0.523267	0.109531	4.777341	1.71E-05	005x250	0.0085
MEST23-C11	0.573196	0.063923	8.966934	2.12E-11	005x250	8.42E-08

EST	Estimate	StdErr	tValue	Raw p	Upregulated	FDR p ≤ 0.05
614070D12.y1	0.59135	0.138668	4.2645	0.000158	005x250	0.034177
MEST11-G04	0.624494	0.155346	4.02002	0.000258	005x250	0.04655
MEST21-F01	0.624565	0.14166	4.408888	7.33E-05	005x250	0.022461
486024D08.x1	0.711013	0.165823	4.287773	0.000148	005x250	0.032551
614074A04.x3	0.754477	0.187856	4.01624	0.000288	005x250	0.047603
486049A11.x4	0.78934	0.192215	4.106555	0.000221	005x250	0.042371
MEST15-E12	0.836131	0.148284	5.63873	1.78E-06	005x250	0.001524
606068H01.y1	0.850357	0.213718	3.978875	0.000283	005x250	0.047603
707020F12.y1	0.902309	0.092347	9.770832	2.23E-12	005x250	1.33E-08
MEST59-G12	0.989471	0.165532	5.977517	5.08E-07	005x250	0.00055
MEST100-A07	1.008941	0.179224	5.629491	2.38E-06	005x250	0.00177
605005D02.y2	1.125284	0.197684	5.692333	1.79E-06	005x250	0.001524
614103H03.x1	1.305261	0.304827	4.281974	0.000209	005x250	0.040819
614020G04.y1	1.732192	0.259083	6.685858	1.3E-07	005x250	0.000257
MEST283-D07	1.81338	0.321202	5.645608	4.77E-06	005x250	0.003154
614074F08.y3	1.830211	0.265784	6.886089	5.08E-07	005x250	0.00055

Table 4. Genotype 250x002 vs. 002, significantly differentially expressed genes (without interaction)

EST	Estimate	StdErr	tValue	Raw p	Upregulated	FDR p ≤ 0.05
707090D10.x1	-0.23042	0.047052	-4.89701	1.14E-05	002	0.01947
606002E05.x1	-0.18418	0.038713	-4.75752	1.97E-05	002	0.029397
606009C10.x1	0.337246	0.064074	5.263376	3.27E-06	250x002	0.009738
687042D04.y1	0.357556	0.058661	6.095273	1.79E-07	250x002	0.001068
687066E03.y1	1.247391	0.240192	5.193311	6.79E-06	250x002	0.013491
614032E07.y1	1.717777	0.267895	6.412132	3.31E-07	250x002	0.001314

Table 5. Genotype 250x002 vs. 250, significantly differentially expressed genes (without interaction)

EST	Estimate	StdErr	tValue	Raw p	Upregulated	FDR p ≤ 0.05
MEST42-E11	-0.47622	0.065324	-7.2901	3.35E-09	250	2.85E-06
MEST326-H09	-0.45273	0.117147	-3.86464	0.000354	250	0.032537
614028A05.y1	-0.44739	0.118922	-3.76203	0.000505	250	0.042361
707082E08.x1	-0.41911	0.087919	-4.76693	1.84E-05	250	0.003987
606009C10.x1	-0.31517	0.064074	-4.91883	1.06E-05	250	0.002636
683006E11.x1	-0.2595	0.067495	-3.8448	0.000377	250	0.03373
MEST42-F05	-0.25123	0.054755	-4.58829	3.22E-05	250	0.006
MEST23-A11	-0.24729	0.060369	-4.09631	0.000164	250	0.020174
687042D04.y1	-0.24549	0.058661	-4.18494	0.000121	250	0.016376
606038D02.x1	-0.24191	0.062017	-3.9007	0.000298	250	0.029574
486066H04.x1	-0.23944	0.047564	-5.03416	7.18E-06	250	0.001946
486085H07.x2	-0.23172	0.055818	-4.15132	0.000138	250	0.018049
707056B10.x1	-0.21253	0.04982	-4.26607	0.000101	250	0.01414
614067B12.x1	-0.15563	0.037234	-4.17969	0.000126	250	0.016855
683008D06.x1	0.110813	0.021566	5.138401	5.52E-06	250x002	0.001602
486028G05.x1	0.127345	0.021941	5.803942	4.99E-07	250x002	0.000238
603015F07.x1	0.139761	0.036908	3.786724	0.000425	250x002	0.0364
486093F05.y1	0.149792	0.03937	3.804692	0.000402	250x002	0.035451
486089A05.x1	0.162578	0.04013	4.051288	0.000185	250x002	0.021633
707090G04.x1	0.171461	0.044527	3.850678	0.000355	250x002	0.032537
707059C05.x1	0.184868	0.032505	5.6874	7.5E-07	250x002	0.000331
707056E08.x1	0.198203	0.047821	4.144687	0.000141	250x002	0.018234
486069G09.x1	0.216898	0.059069	3.671969	0.000614	250x002	0.047212
618025B08.x3	0.228326	0.052963	4.311033	8.25E-05	250x002	0.011987
486022D06.x2	0.242644	0.039342	6.16762	1.62E-07	250x002	9.66E-05
MEST73-G12	0.254605	0.047683	5.339498	2.79E-06	250x002	0.000875
486093D03.y1	0.256906	0.04309	5.962031	2.87E-07	250x002	0.000155
486068C07.x1	0.263702	0.067506	3.906338	0.000293	250x002	0.029549
486093A08.y1	0.269831	0.048117	5.607824	1.05E-06	250x002	0.000447
945004G03.X1	0.280869	0.07268	3.864449	0.000355	250x002	0.032537
606067F06.y1	0.281863	0.075518	3.732376	0.000521	250x002	0.042755
MEST81-E01	0.282539	0.076258	3.705034	0.000587	250x002	0.045416
496022B11.x1	0.284421	0.05927	4.798751	1.72E-05	250x002	0.003796
486068D09.x1	0.300764	0.055452	5.423834	1.88E-06	250x002	0.000652
486068B10.y1	0.301808	0.038635	7.811873	4.24E-10	250x002	4.59E-07
MEST257-B03	0.318256	0.087186	3.650322	0.000667	250x002	0.049986
606054C07.x2	0.322299	0.081992	3.93086	0.000296	250x002	0.029574
606020E08.x1	0.329321	0.07083	4.64948	2.93E-05	250x002	0.005531
606021A06.x2	0.333126	0.039448	8.444698	4.73E-11	250x002	8.1E-08
618004F11.x1	0.335058	0.068639	4.881477	1.49E-05	250x002	0.003415
486022E05.x2	0.340376	0.085794	3.96736	0.000247	250x002	0.026462
486019D06.x5	0.359189	0.074281	4.835549	1.41E-05	250x002	0.003288
614028A08.y1	0.362525	0.08531	4.249525	0.000101	250x002	0.01414

EST	Estimate	StdErr	tValue	Raw p	Upregulated	FDR p ≤ 0.05
614045E12.x4	0.368835	0.077778	4.742143	1.93E-05	250x002	0.004101
707020F12.y1	0.372856	0.095502	3.904171	0.000337	250x002	0.031821
606029C04.x1	0.38947	0.084239	4.623382	3.44E-05	250x002	0.006206
707017E10.x1	0.392573	0.062129	6.318678	9.62E-08	250x002	6.37E-05
605018B04.x1	0.411004	0.043694	9.406325	1.81E-12	250x002	1.08E-08
496004F11.x1	0.415967	0.074796	5.561362	1.39E-06	250x002	0.000552
605018G08.x1	0.430473	0.078238	5.502074	1.51E-06	250x002	0.00058
486036C10.x1	0.434575	0.115544	3.761124	0.000529	250x002	0.043179
707064F09.y1	0.439053	0.07992	5.493672	1.56E-06	250x002	0.00058
MEST15-E12	0.439877	0.081614	5.389741	3.64E-06	250x002	0.001083
687063C12.y1	0.465242	0.103921	4.476867	4.96E-05	250x002	0.008211
MEST23-C11	0.491426	0.066732	7.364139	3.84E-09	250x002	3.05E-06
486024D03.x1	0.503595	0.083368	6.040655	2.17E-07	250x002	0.000123
614014C02.y1	0.525699	0.142797	3.681445	0.000656	250x002	0.049434
707041F02.x1	0.526922	0.126723	4.15806	0.00017	250x002	0.020685
MEST15-B04	0.533646	0.128907	4.13978	0.00018	250x002	0.021212
MEST403-B05	0.544047	0.130645	4.164322	0.000173	250x002	0.020799
496030G01.x1	0.564386	0.114464	4.930681	1.16E-05	250x002	0.002815
MEST21-F01	0.571427	0.145341	3.931631	0.000318	250x002	0.030529
614056D08.x1	0.618065	0.162958	3.792793	0.000534	250x002	0.043251
MEST15-E12	0.636243	0.143707	4.427373	7.8E-05	250x002	0.011461
707020H07.y1	0.673115	0.170179	3.955336	0.000313	250x002	0.030296
945003B11.X3	0.696768	0.183629	3.794442	0.000547	250x002	0.043407
618046D07.y1	0.707367	0.186203	3.798892	0.000452	250x002	0.038456
MEST23-E01	0.720487	0.17999	4.002929	0.00031	250x002	0.030296
486049A11.x4	0.727238	0.180155	4.036735	0.000271	250x002	0.027563
605005D02.y2	0.735465	0.191256	3.845454	0.000472	250x002	0.039873
614067C02.x1	0.769396	0.166596	4.618338	3.36E-05	250x002	0.006161
614069D02.y1	0.772771	0.105532	7.322606	5.8E-09	250x002	4.31E-06
486024D08.x1	0.781024	0.178679	4.371101	0.000116	250x002	0.01607
MEST20-H012	0.809042	0.168256	4.808395	2.07E-05	250x002	0.004331
496034E08.x1	0.844738	0.208387	4.053699	0.000249	250x002	0.026462
614088B08.y1	0.854704	0.208015	4.108861	0.000191	250x002	0.022118
614012G11.x1	0.905861	0.218681	4.142389	0.000258	250x002	0.026497
MEST59-G12	0.956381	0.160886	5.944478	5.66E-07	250x002	0.000259
486024F06.x1	1.040561	0.19939	5.218729	7.16E-06	250x002	0.001946
614020G04.y1	1.845834	0.277601	6.649233	1.45E-07	250x002	9.07E-05
496026A12.x1	1.875816	0.250598	7.485348	0.000672	250x002	0.050043
614103H03.x1	1.877036	0.308044	6.093393	1.65E-06	250x002	0.000597
614074F08.y3	1.877238	0.263941	7.112353	3.03E-07	250x002	0.000157

Table 6. Genotype 301x005 vs. 005, significantly differentially expressed genes (without interaction)

EST	Estimate	StdErr	tValue	Raw p	Upregulated	FDR p ≤ 0.05
618028A09.x1	-0.3746	0.067521	-5.54791	1.37E-06	005	0.001084
MEST28-H11	-0.36038	0.082419	-4.37254	7.18E-05	005	0.023019
486101E10.x1	-0.34122	0.085256	-4.0023	0.000243	005	0.048111
603020G12.x1	-0.3399	0.083414	-4.07487	0.000176	005	0.04221
MEST28-E05	-0.33802	0.083927	-4.02756	0.0002	005	0.046427
618034E12.x1	-0.33364	0.08112	-4.11288	0.000178	005	0.04221
MEST370-H12	-0.28001	0.058947	-4.7502	2.02E-05	005	0.008567
606044D01.x1	-0.25669	0.059959	-4.28119	9.09E-05	005	0.027646
614070D09.x1	-0.17602	0.041245	-4.26762	9.76E-05	005	0.028239
707059C05.x1	0.138422	0.032505	4.25851	9.52E-05	301x005	0.028239
605018B04.x1	0.209604	0.043694	4.797048	1.6E-05	301x005	0.007925
606021A06.x2	0.219022	0.039448	5.552181	1.2E-06	301x005	0.001018
606058B03.x2	0.251521	0.063665	3.950722	0.000255	301x005	0.048115
486068B10.y1	0.269003	0.038635	6.962768	8.39E-09	301x005	1.66E-05
486093D03.y1	0.285728	0.04309	6.630926	2.71E-08	301x005	3.57E-05
486093A08.y1	0.28762	0.047247	6.087627	1.98E-07	301x005	0.000181
486069G09.x1	0.288628	0.059069	4.886316	1.23E-05	301x005	0.006655
605018G08.x1	0.294211	0.074364	3.956345	0.000256	301x005	0.048115
486068D09.x1	0.298478	0.055452	5.382616	2.17E-06	301x005	0.001605
486068C07.x1	0.313148	0.067506	4.638811	2.73E-05	301x005	0.010429
486019D06.x5	0.319755	0.074281	4.304676	8.2E-05	301x005	0.025593
MEST177-D11	0.383649	0.09497	4.039664	0.000211	301x005	0.046427
614064H12.y1	0.385582	0.093956	4.103855	0.000222	301x005	0.046927
MEST31-H08	0.446332	0.092204	4.840688	1.44E-05	301x005	0.007419
614069D02.y1	0.452933	0.107662	4.207007	0.000137	301x005	0.036173
614077E11.x3	0.581729	0.140666	4.135535	0.000176	301x005	0.04221
MEST403-B05	0.607793	0.126704	4.796961	2.5E-05	301x005	0.009878
MEST12-C02	0.654394	0.143794	4.550898	5.11E-05	301x005	0.017889
614069C07.y1	0.71187	0.148226	4.802586	2.22E-05	301x005	0.009065
MEST22-G06	0.715701	0.136598	5.239476	4.88E-06	301x005	0.003175
MEST42-E04	0.882338	0.167897	5.255251	4.92E-06	301x005	0.003175
MEST39-A06	0.886767	0.178185	4.976649	1.72E-05	301x005	0.008168
MEST31-C07	0.903649	0.133724	6.757565	7.87E-08	301x005	9.34E-05
614001E05.x1	0.99478	0.237845	4.182476	0.000231	301x005	0.047189
614016G06.y1	1.037815	0.243196	4.267408	0.000122	301x005	0.033686
614063E07.y1	1.121089	0.265678	4.219723	0.000208	301x005	0.046427
614016D07.x1	1.203948	0.265267	4.538621	5.81E-05	301x005	0.019138
614066F07.y1	1.228464	0.299671	4.099377	0.000243	301x005	0.048111
MEST22-A10	1.235411	0.233666	5.28708	6.23E-06	301x005	0.003521
MEST77-F03	1.315786	0.241402	5.450605	5.35E-06	301x005	0.003175
945002F12.X3	1.352803	0.306502	4.413688	0.00022	301x005	0.046927

Table 7. Genotype 301x005 vs. 301, significantly differentially expressed genes (without interaction)

EST	Estimate	StdErr	tValue	Raw p	Upregulated	FDR p ≤ 0.05
614097D07.x1	-1.52378	0.386418	-3.94334	0.000692	301	0.046186
707040G01.x5	-1.17794	0.27023	-4.35905	0.000212	301	0.023854
614048B04.y1	-1.04655	0.190026	-5.50741	1.34E-05	301	0.003826
606045D02.x1	-0.92129	0.194529	-4.73603	4.27E-05	301	0.00838
486018C12.x4	-0.89222	0.239538	-3.72474	0.000687	301	0.046063
614074D08.y3	-0.86598	0.232216	-3.72921	0.000678	301	0.046017
614048B11.x1	-0.80534	0.198577	-4.05555	0.000276	301	0.028355
MEST31-C07	-0.46562	0.126223	-3.6889	0.00076	301	0.048454
707094D05.x2	-0.38569	0.091058	-4.23563	0.000111	301	0.016021
MEST101-C01	-0.37297	0.087504	-4.26227	9.66E-05	301	0.015223
606058B03.x2	-0.36112	0.063665	-5.67225	7.91E-07	301	0.00051
486066D07.x1	-0.35623	0.058559	-6.08338	1.87E-07	301	0.000181
603028B05.x1	-0.35275	0.086439	-4.08087	0.000177	301	0.022412
614010D03.x2	-0.31104	0.081957	-3.79518	0.000422	301	0.035458
486075E05.x1	-0.28363	0.070018	-4.05083	0.000199	301	0.023104
606066C12.y1	-0.20632	0.049028	-4.20813	0.000112	301	0.016021
486092H04.y1	-0.19054	0.038281	-4.97748	8.7E-06	301	0.002886
945034E07.Y1	-0.18652	0.049456	-3.77146	0.000462	301	0.037767
606028A01.x1	-0.17681	0.044748	-3.95115	0.000254	301	0.027036
MEST254-H06	-0.17278	0.044833	-3.85381	0.000366	301	0.033572
618013C05.x1	-0.16961	0.047021	-3.60713	0.000736	301	0.048253
486021F12.x3	-0.16667	0.043957	-3.79155	0.000419	301	0.035458
606062C01.x1	-0.16526	0.046081	-3.58633	0.000784	301	0.049427
606014D09.x1	-0.152	0.037493	-4.05419	0.000184	301	0.022412
MEST44-E04	-0.14911	0.03462	-4.30712	8.13E-05	301	0.013681
606065B09.x1	-0.12539	0.031626	-3.96479	0.000244	301	0.026184
605015G12.x1	-0.11328	0.026638	-4.25268	9.71E-05	301	0.015223
MEST13-A12	0.115226	0.029989	3.842249	0.000358	301x005	0.033474
707040G12.x5	0.116899	0.030614	3.818526	0.000385	301x005	0.034638
486028G05.x1	0.126492	0.021941	5.765032	5.72E-07	301x005	0.000415
MEST27-A10	0.151027	0.039415	3.831716	0.000377	301x005	0.034152
707090G04.x1	0.160385	0.042006	3.818158	0.000393	301x005	0.034765
MEST24-C11	0.194982	0.050385	3.869863	0.000328	301x005	0.031476
MEST137-B02	0.19596	0.043095	4.547137	3.82E-05	301x005	0.008088
486043A01.x3	0.202046	0.053597	3.769745	0.000448	301x005	0.037384
707061C08.x1	0.202371	0.056203	3.600744	0.00075	301x005	0.048398
486091F11.x1	0.206792	0.050875	4.064745	0.000182	301x005	0.022412
MEST13-C07	0.210891	0.058399	3.611212	0.000751	301x005	0.048398
486066H04.x1	0.211502	0.047564	4.446679	5.15E-05	301x005	0.009457
486047G03.x3	0.243177	0.051245	4.745353	2.05E-05	301x005	0.00542
486024H12.x1	0.253141	0.065471	3.866453	0.000345	301x005	0.032568
614074A05.x3	0.256357	0.050998	5.026838	8.03E-06	301x005	0.00281
614083E04.y1	0.257371	0.0445	5.783569	5.36E-07	301x005	0.000415

EST	Estimate	StdErr	tValue	Raw p	Upregulated	FDR p ≤ 0.05
603030C03.x1	0.264508	0.062036	4.263794	9.61E-05	301x005	0.015223
687042D04.y1	0.265025	0.058661	4.517896	4.07E-05	301x005	0.008293
618046D03.y1	0.267368	0.067031	3.988706	0.000226	301x005	0.024738
MEST21-G10	0.290008	0.074733	3.880598	0.000345	301x005	0.032568
MEST21-A04	0.290896	0.076146	3.82024	0.000415	301x005	0.035458
614020G04.y1	0.29796	0.080344	3.708531	0.00057	301x005	0.04205
606044D01.x1	0.307798	0.060484	5.088936	6.22E-06	301x005	0.002408
606025C08.x1	0.312551	0.079623	3.925374	0.000282	301x005	0.028499
496024C07.x1	0.315154	0.077453	4.068977	0.000179	301x005	0.022412
606029C04.x1	0.315284	0.083504	3.775671	0.000485	301x005	0.038573
MEST67-G12	0.338617	0.083761	4.042639	0.000195	301x005	0.023067
MEST33-C03	0.344291	0.075415	4.565301	3.72E-05	301x005	0.008088
618002E08.x2	0.355073	0.074459	4.768704	1.83E-05	301x005	0.004941
MEST14-G03	0.361051	0.065722	5.493636	1.65E-06	301x005	0.00087
MEST21-G07	0.365782	0.099122	3.690225	0.000592	301x005	0.042124
605013D03.x1	0.367275	0.083002	4.424877	6.27E-05	301x005	0.011027
603016C07.x1	0.369454	0.082016	4.504665	4.53E-05	301x005	0.008627
606059C04.x2	0.371315	0.097241	3.818519	0.0004	301x005	0.03491
MEST61-B07	0.372754	0.082253	4.53177	4.29E-05	301x005	0.00838
614045E12.x4	0.37369	0.077778	4.804553	1.56E-05	301x005	0.00432
606009G12.x1	0.378228	0.076364	4.952965	1.03E-05	301x005	0.003318
606009C10.x1	0.380416	0.064074	5.937127	3.13E-07	301x005	0.000279
606054C07.x2	0.405048	0.077873	5.201398	4.94E-06	301x005	0.001979
MEST18-C05	0.408481	0.096462	4.234621	0.000118	301x005	0.016343
MEST111-F08	0.415072	0.109554	3.788739	0.000456	301x005	0.03756
486092F07.y1	0.415252	0.099837	4.159281	0.00015	301x005	0.019742
618001A08.x2	0.420061	0.112787	3.724363	0.000577	301x005	0.04205
606055A04.x2	0.451046	0.098837	4.563548	4.33E-05	301x005	0.00838
614090D10.x1	0.451466	0.123466	3.656603	0.000706	301x005	0.04681
496026C08.x1	0.453612	0.122792	3.694153	0.000585	301x005	0.04205
606035D08.x1	0.466418	0.125279	3.72303	0.000526	301x005	0.040163
MEST13-G06	0.494037	0.098153	5.033326	8.23E-06	301x005	0.00281
614001C05.x1	0.51373	0.130055	3.950082	0.000301	301x005	0.029323
MEST31-D04	0.513741	0.104898	4.89753	1.35E-05	301x005	0.003826
MEST19-E10	0.514583	0.126876	4.055792	0.000218	301x005	0.024115
MEST29-A03	0.526091	0.14106	3.729554	0.000624	301x005	0.04267
MEST99-F07	0.529486	0.128848	4.109402	0.000197	301x005	0.023104
486036C10.x1	0.546375	0.11642	4.693127	2.99E-05	301x005	0.006679
496030C04.x1	0.627867	0.154824	4.055361	0.000212	301x005	0.023854
618033F09.x1	0.628397	0.170332	3.689246	0.000604	301x005	0.04267
603014E11.x1	0.672992	0.171886	3.915341	0.00029	301x005	0.029064
MEST8-E01	0.677336	0.180264	3.757473	0.000492	301x005	0.038573
MEST253-B10	0.714467	0.143806	4.968268	1.31E-05	301x005	0.003826
MEST19-G10	0.717466	0.185906	3.859297	0.000416	301x005	0.035458

EST	Estimate	StdErr	tValue	Raw p	Upregulated	FDR p ≤ 0.05
606027E05.x1	0.720755	0.193812	3.718831	0.000614	301x005	0.04267
MEST100-A07	0.753606	0.163552	4.607747	5.21E-05	301x005	0.009457
618028A09.x1	0.834437	0.069301	12.04069	8.05E-16	301x005	1.03E-11
618009D01.x1	0.86458	0.141794	6.097419	6.44E-07	301x005	0.00044
603044E07.x1	0.88709	0.157902	5.61798	1.9E-06	301x005	0.00096
MEST19-G09	0.984851	0.095097	10.35624	2.24E-13	301x005	6.51E-10
687066C08.x1	0.98965	0.21675	4.565861	4.88E-05	301x005	0.009127
MEST43-B01	1.069437	0.191962	5.571082	2.04E-06	301x005	0.000987
614074F08.y3	1.424367	0.270841	5.259063	2.46E-05	301x005	0.005708
MEST283-D07	1.461973	0.352471	4.147778	0.000282	301x005	0.028499
614020G04.y1	1.887605	0.245881	7.676902	7.71E-09	301x005	1.12E-05
614073F08.x1	2.040266	0.469222	4.348186	0.000498	301x005	0.038573

Table 8. 99 common genes between at least two inbred-hybrid comparisons

EST	Common between comparisons
486019D06.x5	250x002_250/301x005_005
486022E05.x2	005x250_250/250x002_250
486024D08.x1	005x250_250/250x002_250
486024F06.x1	005x250_005/250x002_250
486024H12.x1	002x301_301/301x005_301
486049A11.x4	005x250_250/250x002_250
486066D07.x1	002x301_301/301x005_301
486066H04.x1	250x002_250/301x005_301
486075E05.x1	002x301_301/301x005_301
486085H07.x2	005x250_250/250x002_250
486089A05.x1	002x301_301/250x002_250
486091F11.x1	002x301_301/301x005_301
486092H04.y1	005x250_005/301x005_301
486093A08.y1	250x002_250/301x005_005
496024C07.x1	002x301_301/301x005_301
496030G01.x1	002x301_301/250x002_250
603030C10.x1	002x301_301/301x005_301
605005D02.y2	005x250_250/250x002_250
605018G08.x1	250x002_250/301x005_005
606020E08.x1	005x250_250/250x002_250
606028A01.x1	005x250_005/301x005_301
606044D01.x1	301x005_301/301x005_005
606054C07.x2	005x250_250/301x005_301
606055H07.x2	002x301_301/005x250_005
606066C12.y1	005x250_005/301x005_301
614010D03.x2	005x250_005/301x005_301
614032E07.y1	005x250_005/250x002_002
614066F07.y1	005x250_005/301x005_005
614067C02.x1	002x301_301/250x002_250
614069C07.y1	005x250_005/301x005_005
614069D02.y1	250x002_250/301x005_005
614083E04.y1	002x301_301/301x005_301
614103H03.x1	005x250_250/250x002_250
618004F11.x1	002x301_301/250x002_250
618046D03.y1	002x301_301/301x005_301
687062G06.x1	002x301_301/005x250_005
687066C08.x1	002x301_301/301x005_301
707017E10.x1	005x250_250/250x002_250
707020F12.y1	005x250_250/250x002_250
707056E08.x1	002x301_301/250x002_250
707059C05.x1	250x002_250/301x005_005
707061C08.x1	002x301_301/301x005_301
707064F09.y1	002x301_301/250x002_250

EST	Common between comparisons
707066F01.y1	002x301_301/005x250_005
707082E08.x1	005x250_005/250x002_250
MEST100-A07.T3	005x250_250/301x005_301
MEST101-C01.T3	002x301_301/301x005_301
MEST13-G06.T3	002x301_301/301x005_301
MEST14-G03.T3	002x301_301/301x005_301
MEST177-D11.T3	005x250_005/301x005_005
MEST18-C05.T3	002x301_301/301x005_301
MEST19-E10.T3	002x301_301/301x005_301
MEST19-G09.T3	002x301_301/301x005_301
MEST20-H12.T3	002x301_301/250x002_250
MEST21-F01.T3	005x250_250/250x002_250
MEST23-C11.T3	005x250_250/250x002_250
MEST24-C11.T3	002x301_301/301x005_301
MEST253-B10.univ	002x301_301/301x005_301
MEST257-B03.univ	002x301_301/250x002_250
MEST283-D07.T3	005x250_250/301x005_301
MEST31-D04.T3	005x250_250/301x005_301
MEST403-B05.univ	250x002_250/301x005_005
MEST42-E04.T3	005x250_005/301x005_005
MEST43-B01.T3	002x301_301/301x005_301
MEST59-G12.T3	005x250_250/250x002_250
MEST67-G12.T3	005x250_005/301x005_301
MEST73-G12.T3	005x250_250/250x002_250
MEST77-F03.T3	005x250_005/301x005_005
MEST12-C02.T3	002x301_301/005x250_005/301x005_005
486028G05.x1	005x250_250/250x002_250/301x005_301
486068B10.y1	005x250_005/250x002_250/301x005_005
486068C07.x1	005x250_005/250x002_250/301x005_005
486068D09.x1	005x250_005/250x002_250/301x005_005
486069G09.x1	005x250_005/250x002_250/301x005_005
486093D03.y1	005x250_005/250x002_250/301x005_005
496022B11.x1	002x301_301/005x250_250/250x002_250
603016C07.x1	002x301_301/250x002_250/301x005_301
605018B04.x1	005x250_250/250x002_250/301x005_005
606014D09.x1	005x250_005/005x250_250/301x005_301
606058B03.x2	005x250_005/301x005_301/301x005_005
614045E12.x4	002x301_301/250x002_250/301x005_301
614070D09.x1	005x250_005/005x250_250/301x005_005
614074A05.x3	005x250_005/005x250_250/301x005_301
618013C05.x1	002x301_301/005x250_250/301x005_301
618028A09.x1	002x301_301/301x005_301/301x005_005
687042D04.y1	250x002_250/250x002_002/301x005_301
707090D10.x1	005x250_005/005x250_250/250x002_002

EST	Common between comparisons
707090G04.x1	005x250_250/250x002_250/301x005_301
MEST137-B02.T3	005x250_005/005x250_250/301x005_301
MEST15-E12.T3	005x250_005/005x250_250/250x002_250
MEST27-A10.T3	005x250_005/005x250_250/301x005_301
MEST31-C07.T3	005x250_005/301x005_301/301x005_005
MEST31-H08.T3	002x301_301/005x250_005/301x005_005
486036C10.x1	002x301_301/005x250_005/250x002_250/301x005_301
606008C10.x1	005x250_005/250x002_250/250x002_002/301x005_301
606021A06.x2	002x301_301/005x250_005/250x002_250/301x005_005
606029C04.x1	002x301_301/005x250_250/250x002_250/301x005_301
614020G04.y1	002x301_301/005x250_250/250x002_250/301x005_301
614074F08.y3	002x301_301/005x250_250/250x002_250/301x005_301

Table 9. Map positions for 20 significantly differentially expressed ESTs from the SCMV experiment

EST	Map positions			
	EST	GSS	EST TUG	Maize Nucleotide
605018B03.x1			bin 1 (1.02) bin 7 (7.02)	bin 6 (6.0)
605018B04.x1				
606007B06.x1				
606021F11.x2		bin 3 (3.09)		
614013G06.x1				
614044F12.x4	bin 8 (8.04)	bin 8 (8.04)		
945031C10.X1				
949062B09.y1				
MEST12-E11.T3				
MEST19-G10.T3	bin 10 (10.04)		bin 10 (10.04)	
MEST22-A03.T3				
MEST24-E10.T3				
MEST24-G11.T3				
MEST40-B08.T3				
MEST40-G05.T3		bin 8 (8.06)		
MEST41-B03.T3				
MEST63-E12.T3				
MEST67-A07.T3			bin 1 (1.06)	
MEST82-F04.T3	bin 10 (10.04)		bin 10 (10.04)	
MEST333-H11.T3				

Table 10. Map positions for 7 RGA sequences from CAU collection

RGAs	Map positions
Zm06_09h07_R	1.07; 2.04; 2.09; 4.08; 10.04
PAC000000001182	6.07
946126A02.y1	8.05; 8.06
1091032B12.y1 a	8.05; 8.06
1091032B12.y1 b	8.05; 8.06
za72g09.b50	3.04
946063C12.y1	8.05; 8.06
exon 1 (eIF3E barley gene)	

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