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Genomic regions under selection in crop-wild hybrids of lettuce: implications for crop breeding and environmental risk assessment

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Summary

The introduction of genetically modified (GM) crops has raised public concern about the potential ecological risks of transgene escape from crops to their wild relatives. A transgene that would give crop–wild hybrids a higher fitness might cause an increased invasiveness of the recipient plants, which could lead to increased costs for weed control, a displacement of some of the original genetic diversity in the wild relative (including crop land races), or even loss of native species from the local flora. On the other hand, GM crops can provide higher yields and reductions in the use of fertilizers, fungicides, pesticides, and water usage. The ongoing debate regarding risks has led to stringent regulations surrounding GM crops, in which companies have to perform an intensive Environmental Risk Assessment (ERA) before a GM crop is allowed to be commercially cultivated.

In recent years, it has become clear that many crops hybridize with their wild relatives at least in some part of their geographic distribution. Moreover, increased weediness has been observed for several wild relatives that received conventional crop alleles through hybridization, although this effect has until now not been observed as a result of a transgene escape. If gene flow between crops and their wild relatives is possible, the key factor governing the spread of a transgene after a hybridization event is the fitness of hybrid individuals in the natural environment.

In addition to the fitness effect of the transgene itself, the location of the transgene and the fitness effects of the surrounding genes can also be important because of linkage drag (= hitchhiking) effects. A transgene located in close linkage with a crop gene or genomic block that contributes positively to crop–wild hybrid fitness will have a higher chance of introgressing into the wild population. Alternatively, placing a transgene in close linkage to regions or crop alleles that confer a negative fitness effect in the wild habitat may lower the chance of a spread of the transgene; this method therefore has been suggested as a potential Transgene Mitigation (TM) strategy. In order to know whether this a feasible approach it is essential that we know more about the effects of genes of domesticated crops after entering a wild genetic background. In this thesis, we use Quantitative Trait Loci (QTL) analyses to pinpoint such genomic regions under selection and the traits, which are associated with these regions. Based on a series of greenhouse and field experiments, we performed QTL analyses on fitness and fitness-related traits under general conditions as well as under abiotic stress and competition, aiming to investigate the likelihood of introgression of crop alleles to wild relatives and the applicability of TM strategies.

To study if different genomic regions have different introgression chances, we use lettuce as a non-transgenic crop–wild model system. The crop lettuce (*Lactuca sativa* L.) is cultivated worldwide and its distribution overlaps with the geographic distribution of its wild relative prickly lettuce (*Lactuca serriola* L.). Cultivated and wild lettuce species are fully interfertile, without any known pre- or post-zygotic barriers. Previous research has indicated that crop–wild hybrids showed increased vigor compared to the wild relative, lasting up to four generations. In recent years, a large set of genotyped Recombinant Inbred Lines (RILs) from a cross between cultivated Iceberg lettuce (*L. sativa* cv. Salinas) and a Californian *L. serriola* plant (UC96US23) has become available, making it possible to test the same genotypes several times in controlled experiments and under field conditions. In addition, we studied early generation hybrids, i.e., Backcross lines selfed for one generation (BC_1S_1) from a cross between the cultivar *L. sativa* cv. Dynamite (Butterhead) and a *L. serriola* collected near the town of Eys, The Netherlands. This combination of greenhouse and field studies, different parental lines, as well as early- and late-generation hybrids, allowed us to perform an extensive analysis of genomic selection patterns

Summary

and associated traits that will affect the likelihood that future transgenes may introgress to wild relatives.

In a first greenhouse experiment, we investigated the genetic architecture of lettuce domestication using RILs, by measuring traits during the entire life cycle of the plants. We found that a few genomic regions with major QTL plus various intermediate QTL control the difference between wild and cultivated Crisphead lettuce. Domestication traits are generally believed to impose negative fitness effects on hybrid individuals in the wild habitat, because they were originally selected for a good performance in an agricultural setting. Indeed, two main regions with clusters of QTL were identified with such negative effects, one on linkage group (LG) 3 where the crop allele induced broader leaves and lower seed output and another on LG7 controlling the speed of development, with the crop allele causing a delay in flowering time. In case a transgene would be inserted in one of these regions, background selection on the crop alleles may lead to a reduced fitness of hybrids with the transgene.

To verify if these same genomic regions could be detected under field conditions, we set up an experiment with the same RILs at two different sites. We performed a QTL analysis on a broad set of fitness and fitness-related traits at different life stages relevant to the success of *Lactuca* hybrids in the field and identified two main genomic fitness QTL. We confirmed the region on LG7 as a region where the crop genomic background conferred a selective disadvantage to crop-wild hybrids, mainly through delaying flowering. However, we also detected a fitness QTL on LG5 where the crop allele consistently conferred a selective advantage by inducing a higher seed output, an effect that was not identified in the greenhouse. Comparison of traits measured in the field and in the previous greenhouse study showed considerable differences in QTL patterns.

In addition to following plants through their entire life cycle under general greenhouse or field conditions, we also performed a series of experiments in which plants were exposed to abiotic stress and competition. It has been argued that especially abiotic tolerance transgenes could potentially contribute to a higher fitness or competitiveness of a wild relative, which increases the probability of introgression of genes from crops to wild relatives. Plants were subjected to drought, low nutrients, salt stress, and above ground competition. Above ground biomass at the end of the rosette stage was used as a proxy for the performance of plants under a particular stress. Here, we detected a mosaic of abiotic stress QTL distributed over the entire genome with little overlap between QTL from different stresses. Those few QTL clusters that were identified reflected general growth characteristics rather than specific stress responses and co-located with the clusters found for leaf shape on LG3 and flowering time on LG7. Furthermore, the field stress treatments were more correlated to greenhouse competition treatments than to the other greenhouse stress experiments, suggesting that competition rather than abiotic stress was a major factor in the field. Therefore, the fate of stress tolerance transgenes under field conditions cannot easily be predicted from controlled greenhouse experiments under abiotic stress.

At the same two sites, we also studied Backcross lines of a cross between a crop *L. sativa* cv. Dynamite (Butterhead) and a Dutch wild relative *L. serriola* (Eys). We performed QTL analyses on the same set of fitness(-related) traits and estimated the fitness distribution of these early- and late-generation hybrid lines. For the BC lines, we also identified the region on LG7 as a region where the crop genomic background consistently conferred a selective disadvantage. However, the RIL fitness QTL on LG5 (crop genomic background selectively advantageous) was not present in the BC crop-wild hybrids. Moreover, in BC lines we identified an additional

fitness QTL on LG6, where again the crop allele conferred a selective disadvantage by inducing delayed flowering and lower survival. Fitness distributions indicated that, although the average fitness of the hybrid offspring was lower than the fitness of the wild parent, several RILs and BC₁ hybrid lineages outperformed the wild parent in both field environments.

In conclusion, the results of this thesis show that the probability of introgression of a putative transgene to wild relatives indeed depends strongly on the insertion location of the transgene. The study of genomic selection patterns can identify crop genomic regions under negative selection in multiple environments and in several cultivar–wild crosses, which could be applicable to TM strategies. For lettuce, a fitness QTL region was identified on LG7 where the crop allele conferred a selective disadvantage through delayed flowering, lower survival, and a lower fitness and possibly through a lower competitive ability. This crop genomic background is likely deleterious in the wild habitat, especially in environments with high density competition and a seasonal flowering period. However, QTL results were also (partly) cultivar specific, so that implementation in ERA will still need to be on a case-by-case basis, which decreases its general applicability.

In addition, our results show that it is more informative to identify specific genomic regions under selection than to estimate average hybrid fitness. Fitness distributions indicated there is a high likelihood in lettuce for novel crop–wild hybrids to arise that have a higher fitness than the wild parent even though the average fitness of the hybrid offspring was lower. This provides an avenue for introgression of crop alleles into the wild population. A mitigation strategy based solely on placing a transgene close to a major negatively selected crop gene could have a huge quantitative effect by reducing the chances of introgression, but it would be difficult to design a strategy that would prevent it with certainty. It is commendable to evaluate the remaining risk in the perspective of the potential consequences an escape would have for the recipient environment, as well as the foreseen benefits of the GM crop.