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Projekttitel

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Projekttitel på svenska enligt projektansökan: Identifiera genetiska regioner associerade med låg kadmiumackumulering i havrekorn

Projekttitel på engelska enligt projektansökan: Identifying genetic regions associated with low Cadmium accumulation in oat grains

Projektledare: Fluturë Novakazi (until Decemeber 2022), Rodomiro Ortiz

Författare till rapporten: Fluturë Novakazi

Fakultet: Department of Plant Breeding

Institution:

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Projektpartners: Alf Ceplitis (Lantmännen Lantbruk)

Projektsammanfattning

Svenska
Kadmium (Cd) är en giftig tungmetall som kan ansamlas i spannmål, inklusive havre. I takt med att konsumenternas efterfrågan på havrebaserade livsmedel ökar – särskilt för hälsomedvetna och växtbaserade produkter – blir Cd-nivåerna i havre ett växande problem. Regulatoriska gränsvärden är strikta, särskilt för produkter avsedda för barn, vilket gör det viktigt för uppfödare och odlare att vara uppmärksamma på Cd-ansamling i havre.

Vår studie undersökte den genetiska grunden för Cd-ansamling i två havreavelspopulationer som testats under fältförhållanden i södra Sverige. Vi identifierade tre genomiska regioner (QTL) som sannolikt är involverade i att kontrollera Cd-nivåerna i havre. Även om ytterligare forskning behövs för att validera och förfina dessa fynd, pekar resultaten redan på genetisk variation som kan utnyttjas i avelsprogram.

Viktiga budskap:

• **Genetiska skillnader spelar roll**: Även inom elitförädlingsmaterial varierar kadmiumackumuleringen avsevärt. Att välja rätt föräldrar kan bidra till att minska kadmiumnivåerna i framtida sorter.

• **Fältförsök är avgörande**: Kadmiumupptaget påverkas av markegenskaper, väder och växtgenetik. Fleråriga försök är fortfarande en hörnsten för tillförlitlig fenotypning.

• **Använd tillgängliga verktyg**: I takt med att fler genomiska resurser blir tillgängliga för havre kan markörassisterat urval bidra till att påskynda utvecklingen av kultivarer med lågt kadmiuminnehåll.

• **Tänk på miljön**: Att hantera jordens pH, organiskt material och gödslingsmetoder kan också bidra till att minska växternas kadmiumupptag.

Framöver bör förädling för låg kadmiumackumulering prioriteras, särskilt eftersom havre fortsätter att komma in på nya livsmedelsmarknader. Våra resultat erbjuder ett första steg mot att identifiera och selektera för egenskaper med lågt kadmiuminnehåll i förädlingsportföljer.

English
Cadmium (Cd) is a toxic heavy metal that can accumulate in cereal grains, including oats. As consumer demand for oat-based foods increases—especially for health-conscious and plant-based products—Cd levels in oats are becoming a growing concern. Regulatory limits are strict, particularly for products intended for children, making it important for breeders and growers to pay attention to Cd accumulation in oat grain.

Our study investigated the genetic basis of Cd accumulation in two oat breeding populations tested under field conditions in southern Sweden. We identified three

genomic regions (QTLs) that are likely involved in controlling Cd levels in oat grain. Although further research is needed to validate and refine these findings, the results already point to genetic variation that can be exploited in breeding programs.

Key takeaway messages:

- **Genetic differences matter**: Even within elite breeding material, Cd accumulation varies significantly. Choosing the right parents can help reduce Cd levels in future varieties.
- **Field testing is essential**: Cd uptake is influenced by soil properties, weather, and plant genetics. Multi-year trials remain a cornerstone for reliable phenotyping.
- **Use available tools**: As more genomic resources become available for oats, marker-assisted selection can help speed up the development of low-Cd cultivars.
- **Mind the environment**: Managing soil pH, organic matter, and fertilization practices can also help reduce plant Cd uptake.

Going forward, breeding for low Cd accumulation should be a priority, especially as oats continue to enter new food markets. Our findings offer a first step toward identifying and selecting for low-Cd traits in breeding pipelines.

Abstract

Cadmium (Cd) is a persistent, non-essential heavy metal that accumulates in agricultural soils and poses serious health risks when entering the food chain, particularly through cereal grains. While Cd uptake, translocation, and accumulation have been widely studied in major cereals like rice and wheat, little is known about the genetic mechanisms governing Cd accumulation in oats (*Avena sativa*). In this study, we investigated the genetic architecture of Cd accumulation in oat grains using QTL mapping in two biparental F_6 populations (SW 130616 × SW 130912 and A7:4 × Galant), evaluated over two years in field trials in Svalöv, Sweden.

QTL mapping was conducted via composite interval mapping using best linear unbiased estimators (BLUEs) as phenotypic input. Three QTL associated with Cd grain content were identified. In the A7:4 × Galant population, significant QTL were found on linkage groups (LG) 13 and 15. LG 15 corresponds to chromosome 1D, with the QTL spanning 295–305 Mbp. In the SW 130616 × SW 130912 population, one suggestive QTL was identified on LG 3, corresponding to chromosome 2D, though it did not reach the significance threshold.

The genetic resolution was limited by moderate population sizes and marker density, which likely hindered the detection of additional QTL. Comparison with prior studies, including an unpublished GWAS conducted in Lantmännen's oat breeding population, revealed no overlap with the loci identified here, suggesting population-specific QTL or non-overlapping segregating alleles. Nevertheless, our findings contribute new insights into the genetic basis of Cd accumulation in oats and provide a foundation for marker-assisted selection in oat breeding programs aimed at reducing Cd levels in the grain to ensure food safety.

Bakgrund

Cadmium (Cd) is a non-essential heavy metal, which is very persistent and therefore considered an environmental contaminant. It can remain in the environment for several decades and accumulates especially in the soil. Cd is mainly released through industrial activities like mining, refining and production of plastic. However, it also enters the soil through fertilizers and plant protection products that contain Cd, sewage sludge and manure (Chunhabundit, 2016).

Cd is highly toxic to all living cells. Plants take up Cd from the soil through the root system. It is then translocated to other plant parts and accumulates in leaves and storage organs (Abedi&Mojiri, 2020). High amounts of Cd affect germination, root elongation, and overall growth in plants. It interferes with respiration and photosynthesis by affecting for example chlorophyll production (Abedi&Mojiri, 2020, Rizwan et al., 2016). Additionally, increased Cd uptake has been linked to increased production of reactive oxygen species (ROS) and an overall imbalance of nutrient uptake (Abedi&Mojiri, 2020, Çatav et al., 2020, Rizwan et al., 2016). Ultimately, it leads to decreased biomass and yield production. In cereal crops, like rice, wheat and oat, accumulation of Cd in the grains is especially problematic, since humans consume cereal products in large quantities. In humans, Cd accumulates mainly in the kidney, leading to kidney damage or even failure. Furthermore, high Cd accumulation in humans has been linked among others to bone demineralisation, as well as to lung, bladder and breast cancer (Chunhabundit, 2016). The EU has limited the maximum Cd in cereal products to 0.05 to 0.2 mg/kg wet weight and to 0.04 mg/kg for cereals intended for baby food production (EU Commission Regulation, 2021).

The Cd content in European soils varies greatly between regions, but increased accumulation is obvious in regions with intensive industrial and agricultural activities (Fig. 1).

Generally, the higher the total amount of Cd in the soil, the more the plant will take up. However, the plant available Cd is affected by the solubility of soil Cd, pH, soil type, clay and organic matter content, soil fertility and environmental conditions, especially precipitation during the growth season, as well as the interplay of these factors (Eriksson et al., 1990a, 1990b, 1996, Ciesliniski et al., 1996, He&Singh, 1993, Liu et al., 2015). Genetic differences in Cd uptake and accumulation between and within species have been reported for several crop species (e.g. Arao et al., 2003, Arao&Ishikawa, 2006, Ciesliniski et al., 1996, Eurola et al., 2003, Florijn&Van Beusichem, 1993, Welch&Norvell, 1999, Yang et al., 1995).

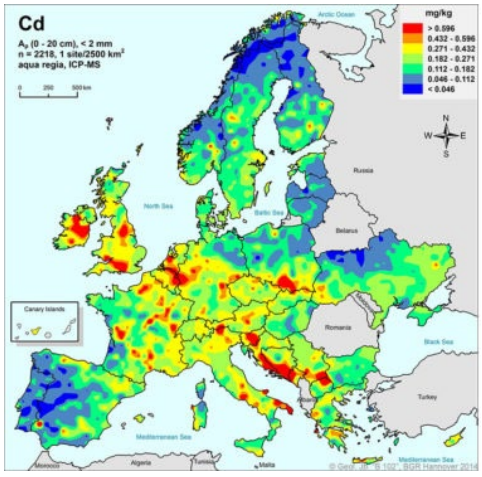

Figure 1 Cadmium content in European soils. (from Reimann et al., 2014)

Plants take up aqueous forms of Cd present in the soil solution. Aqueous species of Cd are Cd^{2+} and complexed or chelated inorganic and organic forms (Welch&Norvell, 1999). Cd^{2+} cations are adsorbed either through the electrochemical potential at the root surface across the plasma membrane, through Cd specific membrane transporters or through transporters specific for other divalent cations like Fe^{2+}, Mg^{2+}or Zn^{2+} (Uraguchi&Fujiwara, 2012, Welch&Norvell, 1999). Several protein families have been reported and identified in several plant species to be involved in Cd uptake by the roots. Amongst these protein families are zinc-regulated transporter (*ZRT*)/ iron-regulated transporter (*IRT*) like proteins (*ZIP*), natural resistance-associated macrophage proteins (*NRAMP*), or low-affinity cation transporters (*LCT*) (reviewed in Abedi&Mojiri, 2020, Uraguchi&Fujiwara, 2012). Cd chelates enter the root through yellow stripe 1-like (*YSL*) proteins (Curie et al., 2009). Once in the root, Cd is translocated into the shoot and leaves via the xylem. A major regulator of root-to-shoot translocation of Cd is the heavy metal ATPase (*HMA*) family, which are tonoplast-localized transporters. Overexpression of *OsHMA3* and *TaHMA3* in rice and wheat respectively, decreased the root-to-shoot translocation significantly by sequestering Cd into the vacuoles (Salt&Wagner, 1993, Sasaki et al., 2014, Ueno et al., 2010, Zhang et al., 2020). Overexpression of the rice membrane protein *OsLCT2* reduced the translocation from the root to the shoot significantly by limiting the amounts of Cd loaded into the xylem (Tang et al., 2021). In a genome-wide transcriptome study on barley plants exposed to high Cd concentration, Kintlová et al. (2021) identified a gene upregulated in root and shoot tissue, *PLANT CADMIUM RESISTANCE 2* (*HvPCR2*). Additionally, they identified four *HvPCR2* in the same region on chromosome 5H. Finally, translocation from the shoot into the grain is related to phloem-mediated Cd transport. In rice, another *LCT* gene, *OsLCT1*, was found to regulate Cd translocation from the shoot to the grain. Loss of function mutants of *OsLCT1* accumulated half as much Cd in the grain as the wild type (Uraguchi et al., 2011).

Genome-wide association studies to identify genomic regions associated with Cd uptake or accumulation were reported manifold for several crop species, e.g. barley (Wu et al., 2015), bread wheat (Hussain et al, 2020, Bin Safdar et al., 2020), durum what (AbuHammad et al., 2016), oil seed rape (Chen et al., 2018) and maize (Zhao et al., 2018). Even though the regulation of Cd uptake and accumulation in the plant is most likely controlled by several genes, major genes and QTL have been identified through QTL mapping in most crop species. Ishikawa et al. (2009) identified two major QTL located on chromosome 2 and 7S associated with increased Cd grain concentration in rice. Three QTL associated with shoot Cd concentration were identified on rice chromosomes 2, 5 and 11 (Ueno et al., 2009). Benitez et al. (2010) identified a major QTL in a soybean RIL population controlling Cd concentration in the seed, which accounted for 57-82% of the genetic variation. In durum wheat a major QTL located on chromosome 5BL was identified in a RIL population explaining 54.3% of the phenotypic variation (AbuHammad et al., 2016). Derakhshani et al. (2020) identified a major and a minor QTL in a barley mapping population on chromosomes 6H and 2H. Subsequent RNA-Seq analysis revealed 16 candidate genes related to Cd tolerance. Several genes have also already been cloned. For example, Benitez et al. (2012) and Ueno et al. (2010) cloned the genes *GmHMA1* in soybean and *OsHMA3* in rice, respectively. Both genes encode P_{1B}-ATPases, and were primarily expressed in the roots.

Syfte

Many studies on Cd uptake, translocation and accumulation have been conducting in major crops like rice and wheat, owing to their economic importance.
Oat (*Avena sativa*) production ranks sixth in worldwide cereal production after maize, rice, wheat, barley, and sorghum, with the European Union being the biggest producer of oats (FAO, 2020). Most of the oat production is used as animal feed. However, oats are rich in dietary fibre (β-glucan) and protein and since the acknowledgement of its health benefits in the 1990s, oat production for human consumption has increased (Ma et al., 2021). With the increased demand of consumers for non-dairy and locally grown products, a market for oat-based products has developed.
Little research has been done in regard to Cd uptake and accumulation in oat grains. Tanhuanpää et al. (2007) identified a major gene for Cd grain accumulation in oats in an F_2 population of a cross between the oat cultivars 'Aslak' and 'Salo' (high Cd accumulator). However, the exact location of the gene could not be determined. To our knowledge, no follow up studies by the authors, no other QTL mapping nor GWAS studies have been published on the subject.
Our aim is therefore, to dissect the genetic architecture underlying Cd accumulation in oat grains in two oat mapping populations employing a QTL mapping approach. A first oat genome is publicly available (Avena sativa – OT3098 v2, PepsiCo, https://wheat.pw.usda.gov/jb?data=/ggds/oat-ot3098v2-pepsico), which will facilitate the identification of genomic regions involved in Cd uptake and translocation as well as the identification of potential candidate genes.

Metod

Plant material
Two bi-parental populations were used in this study. One population was a cross between the two breeding lines SW 130616 x SW 130912 and consisted of 92 individuals. The second population was a cross between the cultivar Galant and the breeding line A7:4 (A7:4 x Galant) and consisted of 93 individuals. Galant is known to accumulate high amounts Cd, but is nonetheless the most popular oat cultivar grown in Sweden. Both populations were advanced to F6 prior to field trials.

Field trials and Cadmium analysis
The two oat populations were grown on Lantmännen's experimental station in Svalöv during the growth seasons of 2022 and 2023. They were sown as head rows in two replicates. For SW 130616 x SW 130912 only 52 lines could be screened in the field and for Cd grain content since for the other lines there was not enough seed available. For A7:4 x Galant 89 lines were grown in the field and analysed for Cd content.
Cd grain content was analysed with an in-house method at Lantmännen using a microwave assisted extraction (MAE) method followed by graphite furnace atomic absorption spectrometry (GFAAS). Of the fine powder, 0.5g as well as 7 mL of concentrated HNO_3, 2 mL of water and 1 mL of H_2O_2 were transferred to a microwave tube. The tubes were slowly heated under pressure (90 bar) to 180°C for 30 min in a microwave oven. After cooling down, the samples were transferred to new tubes and filled up with water to 50 mL. Subsequently, after settling down, 0.2 mL were transferred to a GFAAS and 10 µL of each sample were heated to 1500°C. The amount of Cd is measured through the adsorption level with a lamp

with a Cd specific wavelength. Each sample was measured three times and a mean for each line was calculated.

QTL mapping
All lines including the parents were genotyped with the 7k Oat chip. The two parents SW 130616 and SW 130912 were genotyped with the 8k chip, which is an extension of the 7k chip and includes markers from a new US chip.
As phenotypic input data Best Linear Unbiased Estimators (BLUEs) were calculated for each population across the Cd results for both years.
Map construction and QTL mapping analysis were performed with the R package qtl (Broman et al., 2003). For the QTL mapping a composite interval mapping (CIM) approach was chosen using the Haley-Knott regression and the Haldane function to estimate the genetic distances between linked genes. The significance threshold for significant marker-trait associations was set at LOD = 3.

Resultat och diskussion

Phenotypic results
The two oat bi-parental populations were tested in the field in Svalöv at two years (2022 and 2023). For the cross A7:4 x Galant the lines showed slightly higher Cd grain content values in 2022 than in 2023 (Fig. 2a). For the population SW 130616 x SW 130912 the average Cd grain content was a bit higher in 2023 (Fig. 2b).
There was more variation in Cd grain content in the population SW 130616 x SW 130912, as these lines showed higher Cd grain contents on average compared to population A7:4 x Galant (Fig. 3). This might be due to the fact that the two parents A7:4 and Galant showed lower Cd grain contents, 0.0286 and 0.0688 mg/kg, respectively, compared to the other parents SW 130616 (0.0455 mg/kg) and SW 130912 (0.0805 mg/kg). The reference cultivar Belinda had an average Cd grain content of 0.0557 mg/kg and hence lay between the low and high accumulating parents (Fig. 3).

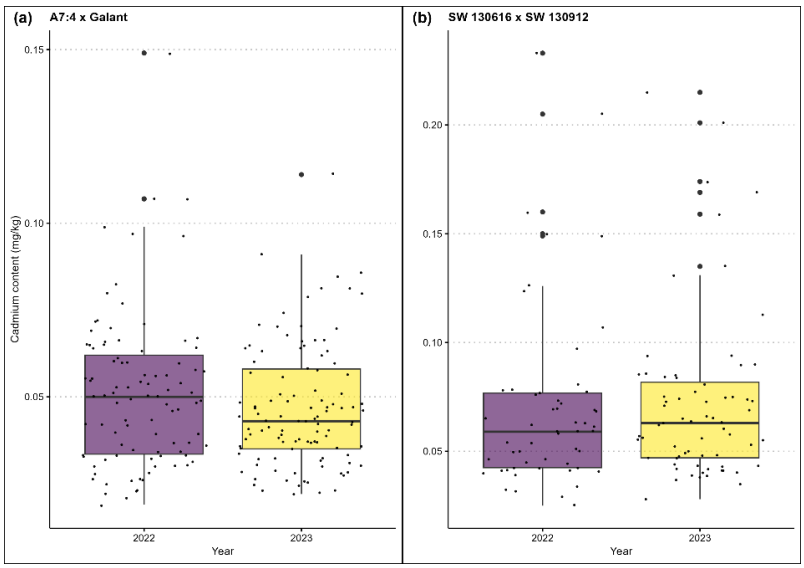

Figure 2 Cadmium grain content of individual oat lines from the two crosses A7:4 x Galant and SW 130616 x SW 130912 grown under field conditions in Svalöv in 2022 and 2023.

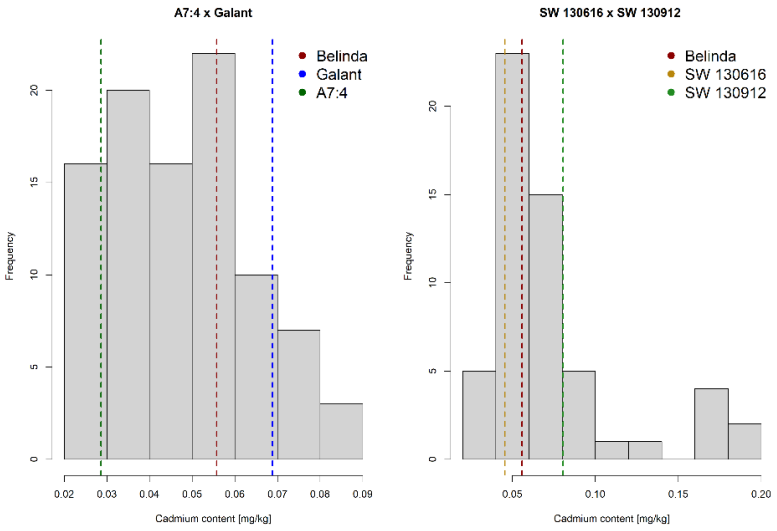

Figure 3 Frequency distribution based on BLUEs (Best Linear Unbiased Estimators) of Cadmium grain content in two oat bi-parental populations, A7:4 x Galant and SW 130616 x SW 130912, grown under field conditions in Svalöv in 2022 and 2023. Dotted lines indicate the values for the reference cultivar Belinda (red) and the four parents, Galant (blue), A7:4 (dark green), SW 130616 (yellow), SW 130912 (green), respectively.

Map construction

To construct the genetic maps, the genotypic data was filtered to match certain quality criteria. These were removing monomorphic SNPs and SNPs with a call rate < 20 %. Genotype call rates were also checked; however, all genotypes passed the cut off rate. This initial filtering left 838 SNPs for population SW 130616 x SW 130912 and 799 SNPs for population A7:4 x Galant to construct the genetic maps.

During the map construction further SNPs were removed leaving 710 and 610 SNP markers for the actual maps of SW 130616 x SW 130912 and A7:4 x Galant, respectively.

The 710 SNP markers for the map of SW 130616 x SW 130912 were grouped into 21 linkage groups (LG) with number of markers per LG varying from 11 to 68 SNPs. The map spanned 735 cM, where individual LG showed lengths between 1.4 to 193.7 cM, with an average spacing of 1.1 cM and a maximum spacing of 68.7 cM between markers. The 610 SNP markers for the map of A7:4 x Galant were grouped into 21 LG with number of markers per LG varying from 10 to 86 SNPs. The total length of the map was 723.8 cM, with an average spacing of 1.2 cM and a maximum spacing of 31.1 cM between markers.

QTL mapping

For population SW 130616 x SW 130912 no significant QTL could be detected. However, there was one peak on LG 3 spanning from 25.85 cM to 35.51 cM with the peak markers located at 28.76 cM (Fig 4). The LOD values for the peak markers were at LOD = 2.81. This linkage group corresponded to oat chromosome 2D. The physical region spanned from 20 Mbp to 135 Mbp. However, the peak markers (in total 16) were located between 20 and 90 Mbp.

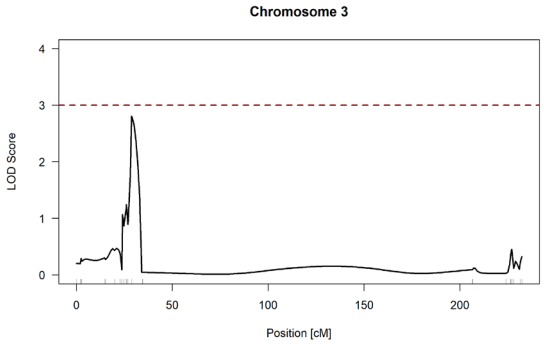

Figure 4 QTL mapping results for population SW 130616 x SW 130912 for linkage group 3. Red dashed line indicates the significance threshold of LOD = 3.

For population A7:4 x Galant two peaks were detected in the QTL mapping analysis. These were located on LG 13 and 15, respectively. The QTL detected on LG 13 mapped between 24.64 and 35.62 cM (Fig. 5). The three peak markers were located at 25.21 to 25.57 cM with LOD values of 3.03.

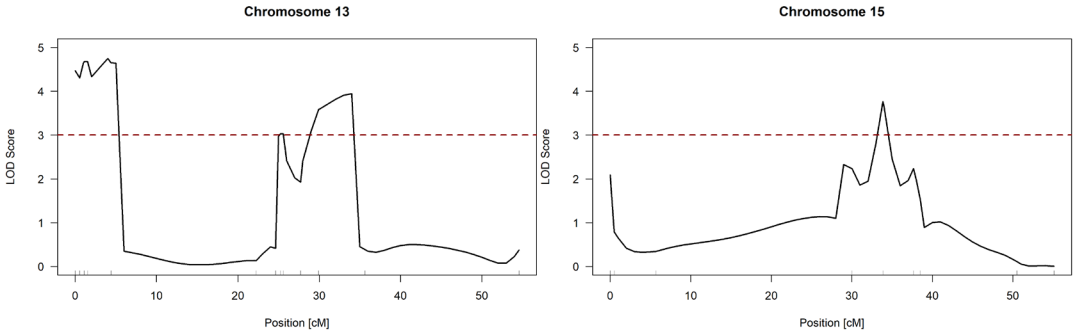

Figure 5 QTL mapping results for population A7:4 x Galant for linkage group 13 and 15. Red dashed line indicates the significance threshold of LOD = 3.

The QTL detected on LG 15 mapped to between 30 and 37.66 cM (Fig. 5). The two peak markers were located at 33.85 cM and had LOD values of 3.77. LG 15 corresponded to oat chromosome 1D and the physical position of the detected QTL was located at 295 to 305 Mbp.

In total three QTL were detected in two bi-parental populations of oat, shedding some light on the genetic architecture of Cd grain accumulation in oats. Tanhuanpää et al. (2007) identified a major gene for Cd grain accumulation, however, they were not able to determine the exact chromosome and location of their QTL. Hence, at this point it is not possible to say, if any on the identified QTL correspond to the major gene identified by Tanhuanpää et al. (2007).

In a genome-wide association study (GWAS) done on Lantmännen's oat breeding population, we identified two QTL associated with Cd grain accumulation located on oat chromosomes 3D and 4A (data unpublished). So far, it does not look like the QTL identified in the current study correspond to the QTL identified in the GWAS. The two bi-parental populations probably do not segregate at the loci found in

GWAS, since the significant markers from the GWAS were removed from the marker panel during filtering for quality criteria.
The data had two constraints to guarantee robust and highly significant results. Firstly, the population size. For population SW 130616 x SW 130912 only 52 lines were tested in the field and were available for phenotyping. For population A7:4 x Galant 89 lines were phenotyped. Secondly, the number of markers (710 and 610) compared to the large genome of oats (~15Gbp) was quite limited, resulting in some cases only in 10 SNPs per chromosome. However, with bigger population sizes and a higher marker saturation, a higher resolution could have been achieved, since more recombination events would have been captured.
Nonetheless, the results show that there are potentially several genomic regions involved in Cd grain accumulation in oats. This should be further explored and investigated so breeders can use this information in their breeding programmes.

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