Arabidopsis semi-dwarfs evolved from independent mutations in GA20ox1, orthologue to green revolution dwarf alleles in rice and barley

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Understanding the genetic bases of natural variation for developmental and stress-related traits is a major goal of current plant biology. Variation in plant hormone levels and signaling might underlie such phenotypic variation occurring even within the same species. Here we report the genetic and molecular basis of semi-dwarf individuals found in natural Arabidopsis thaliana populations. Allelism tests demonstrate that independent loss-of-function mutations at GA5, which encodes a GA 20-oxidase involved in the last steps of gibberellin (GA) biosynthesis, are found in different populations from Southern, Western and Northern Europe, Central Asia and Japan. Sequencing of GA5 identified 21 different loss-of-function alleles causing semi-dwarfness without any obvious general trade-off affecting plant performance traits. GA5 shows signatures of purifying selection, while GA5 loss-of-function alleles can also exhibit patterns of positive selection in specific populations as shown by Fay and Wu's H statistics. These results suggest that antagonistic pleiotropy might underlie the occurrence of GA5 loss-of-function mutations in nature. Furthermore, since GA5 is the orthologue of rice SD1 and barley Sdw1/Denso green revolution genes, this study illustrates the occurrence of conserved adaptive evolution between wild Arabidopsis and domesticated plants.

Arabidopsis natural variation | dwarf accessions | Gibberellin mutants

Bioactive gibberellins (GA) are plant growth regulators involved in important traits such as seed germination, flowering time, flower development, and elongation growth (1). GA biosynthesis and signaling pathways are well defined (1, 2) and have been targeted in crop breeding. Modification of GA pathways was crucial in the green revolution since it conferred semi-dwarfness thus reducing lodging and increasing crop yields (3, 4, 5, 6). Green revolution semi-dwarf varieties in wheat are due to mutations in *DELLA* genes while many short straw rice varieties carry a mutation in the *SD1* (*Semi-Dwarf-1*) locus. This locus codes for *GA 20-oxidase-2,* a GA biosynthesis gene that is also mutated in most modern barley varieties in which the gene was called *Denso* or *Sdw1* (7).

GA 20-oxidases are involved in the later steps of GA biosynthesis and belong to the group of 2-oxoglutarate-dependent dioxygenases that, together with GA 3-oxidases, form biologically active GA (8). *Arabidopsis thaliana* (hereafter referred to as Arabidopsis) has five *GA20ox* paralogous genes. *AtGA20ox-1, -2, -3 and -4* can catalyze the *in vitro* conversion of GA_{12} to GA_9. Therefore, *GA20ox* paralogs might have partial redundant functions (9). However, among paralog genes, only *AtGA20ox-1* (*GA5*), which was cloned on the basis of the *ga5* mutant (10), affected plant height (8).

Natural variation for GA biosynthesis has been previously described in Arabidopsis since the Bur-0 accession carries a loss-of-function allele at *GA20ox4* (9), which does not result in a semi-dwarf phenotype. In addition, genetic variation in *GA1* has been associated with variation in floral morphology (11). Furthermore, the semi-dwarf phenotype (here defined as a plant height shorter than half the size of genetically related individuals) observed in the Kas-2 accession, is due to a recessive allele at the *GA5* locus (12). This latter finding led to the question whether green revolution alleles artificially selected in cereals could also occur in natural populations of the wild species Arabidopsis; and, if so, how many different *GA5* loss-of-function alleles exist, how are they distributed and why do they occur in some populations.

RESULTS

Identification, characterization and geographic distribution of natural *ga5* alleles.

Phenotypic surveys for plant height in world-wide collections of Arabidopsis accessions detected 97 individuals collected in 23 different locations showing semi-dwarf phenotypes. To determine the genetic basis of semi-dwarfness, we carried out allelism tests by crossing at least one semi-dwarf from each population to the recessive *ga5* (L*er*) mutant (13), and to L*er* 'wild type' as control (Fig. 1A and 1B, and SI Appendix Table S1). To discard that GA-biosynthesis mutations other than *GA5* could account for the semi-dwarf phenotypes, we tested the complementation of the

Significance

Semi-dwarf accessions occur at low frequency across the distribution range of *Arabidopsis thaliana* and are mainly mutants of the *GA5 (GA20ox1)* gene, which mutations originate from wild-type alleles still present in the regions where the mutants were found. We identified the causal mutations by allelism tests, and sequencing, and performed a detailed population genetics analysis of this variation. Using Fay and Wu H statistics, we obtained indications for local selection of the dwarf alleles. Importantly, mutants of functional orthologues of this gene have been selected as the so-called green revolution genes in rice and barley, thus indicating that Arabidopsis natural variation can be a source for the identification of useful genes for plant breeding.

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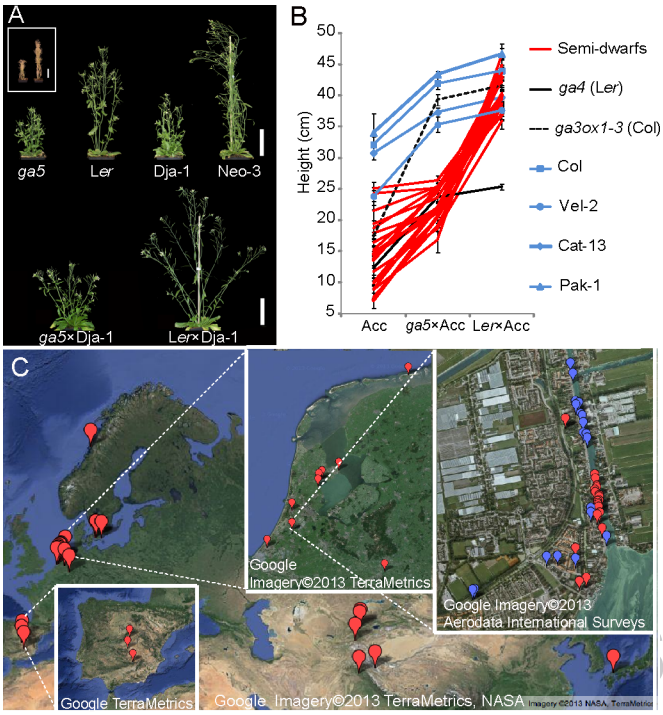

Fig. 1. Semi-dwarf genotypes allelic to *ga5* are present in nature. (A) Allelism test between the semi-dwarf mutant *ga5* (13) and the semi-dwarf central Asian accession Dja-1. Neo-3 (central Asia) shows the phenotype of a functional *GA5*. Pictures were taken two weeks after flowering. On the upper left panel is shown the phenotype of *ga5* and *Ler* at harvesting time. Scale bars, 7 cm. (B) Mean values of stem height ± standard errors in F_1 plants derived from crosses between *ga5* or *Ler* and twenty accessions (Acc) allelic to *ga5* (red), three non-dwarf accessions (Col-0, Pak-1, and Cat-13), two semi-dwarf mutants (*ga4* and *ga3ox1-3*) and one semi-dwarf accession non-allelic to *ga5* (Vel-2). (C) Geographical distribution of semi-dwarf accessions in Europe, Scandinavia and Central Asia. Red marks indicate the location of populations containing semi-dwarf accesions allelic to *ga5*. On the right panel it is shown the detailed local distribution of semi-dwarf (red) and wild-type (blue) individuals found in the OW population from The Netherlands in 2012). Satellite images were obtained from Google maps.

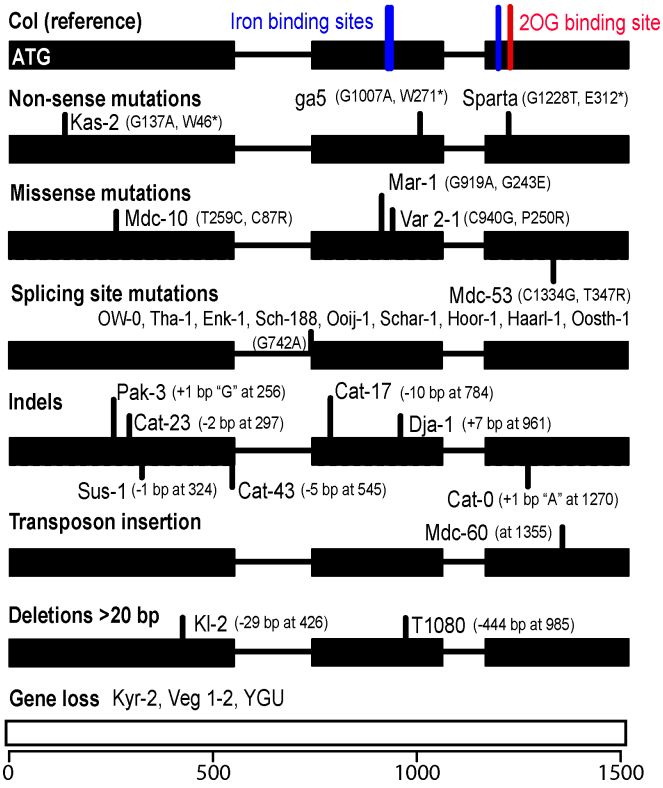

Fig. 2. Natural loss-of-function mutations in the *AtGA20ox1* (*GA5*) gene. The different nature and position of mutations causing *GA5* loss-of-function alleles are shown in each panel. Exons (black boxes) are connected with horizontal lines representing intronic regions of *GA5*. Iron and 2-oxoglutarate binding sites (18) are indicated on top.

ga5 (*Ler*) mutant by two other semi-dwarf mutant alleles also affecting GA biosynthesis: *ga4* (*Ler*), a mutant in the *GA3ox1* gene and *ga3ox1-3* (Col-0) (14) (Fig. 1B and SI Appendix Table S2). *Ler* and Col mutants were used to test background effects. Control F_1 plants derived from crosses between non-dwarf accessions and *ga5* mutant, as well as F_1 plants grown from crosses with other GA mutants were all taller than their corresponding parents. The crosses *ga5* × *ga4* and *Ler* × *ga4* yielded a low height due to the *erecta* mutation which remained recessive in the F_1. In addition, three accessions showing a weaker semi-dwarf phenotype (Nfro, Norway; Kar, Central Asia and Vel, Spain) were not allelic to *ga5*, which indicated that other loci accounted for their plant height phenotype. However, for all the remaining semi-dwarf accessions tested, the F_1 obtained from their cross to *ga5* exhibited the small size of the parents, whereas semi-dwarfism was lost in the cross with *Ler*. This finding confirmed the recessiveness of the semi-dwarf alleles. Therefore, most semi-dwarf accessions were allelic to *ga5*.

To evaluate if there is any general negative pleiotropic effect on plant performance associated with natural *ga5* alleles, we measured several presumably adaptive traits in six wild *ga5* semi-dwarf accessions, as well as in the *ga5* mutants in *Ler* and Col genetic backgrounds (SI Appendix Fig. S1). Consistent with previous studies (8), *ga5* mutants did not differ significantly from their wild-types in the evaluated traits (SI Appendix Fig. S1). However, natural *ga5* accessions strongly differed in flowering time, branch and silique number, indicating the absence of strong *ga5* effects on these traits but the substantial contribution from other genes. Therefore, no major trade-off on silique number, assumed to be a proxy for fitness, was found for these naturally occurring *ga5* alleles.

Semi-dwarf *ga5* accessions were found in 23 different populations distributed in Western Europe, the Iberian Peninsula, Scandinavia, Central Asia and Japan (Fig. 1C, and SI Appendix Table S1). From our analysis, we roughly estimated that, at world-wide scale, the frequency of wild populations containing semi-dwarf accessions allelic to *ga5* was at least 1%. However, these frequencies may be higher, since most populations segregate for *GA5* loss-of-function alleles, we cannot discard that some populations with a limited number of individuals may contain semi-dwarfs at low frequency not represented in the individuals studied. We also found a semi-dwarf frequency of 1% in the Hapmap experimental population consisting of 360 world-wide accessions with empirically reduced population structure (15). However, the frequency of *ga5* semi-dwarf containing populations was not homogeneous throughout the Arabidopsis geographic range since we did not find semi-dwarfs among the many Central and East European accessions studied. By contrast, semi-dwarfism appeared most frequent in Central Asia than elsewhere, since 5 out of the 24 central Asian populations monitored in this and another study (16) carried semi-dwarf individuals (SI Appendix Table S1). A ~2% frequency was estimated for the Iberian Peninsula from the qualitative analysis of the intensive collection (17) used to select the Iberian accessions included in this study. In addition, detailed sampling and analysis of *ga5* semi-dwarfs in The Netherlands indicated a ~5% frequency in this region. Interestingly, Dutch semi-dwarfs seemed to have spread

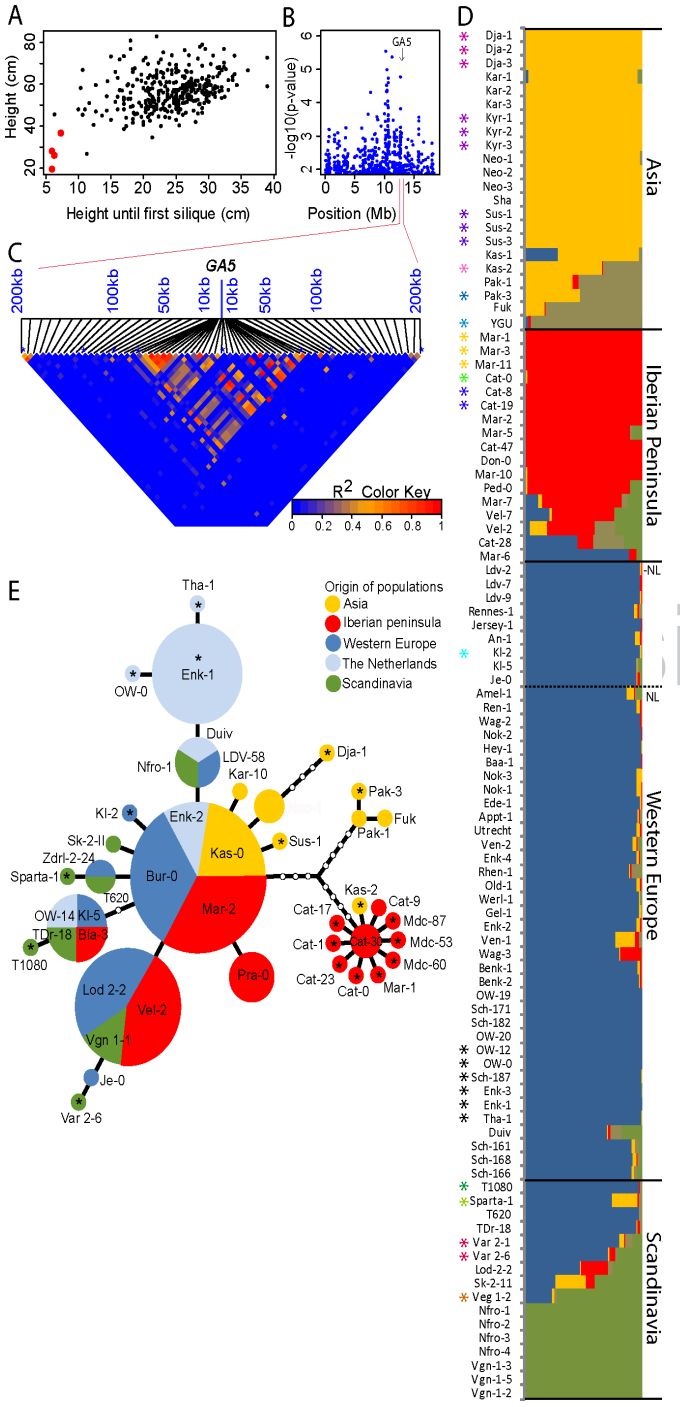

Fig. 3. GWAS analyses, population structure and *GA5* diversity. (A) Correlations between height and height up to first silique. Red dots indicate the values from semi-dwarf accessions. (B) Genome wide association mapping profile for plant height on chromosome 4. The *GA5* position is indicated by an arrow. (C) Linkage disequilibrium 200 kb up and downstream of the *GA5* locus. The heat colour scale represents squared correlation (R^2) between pairs of SNPs. (D) Population structure of 100 accessions including non-dwarf and *GA5* semi-dwarfs collected in different world regions at K=5. Colored asterisks indicate accessions carrying different *GA5* loss-of-function alleles. (E) *GA5* haplotype network. Haplotypes are represented by circles with size proportional to the number of populations containing that haplotype. Each node represents a single mutation.

mainly in the west of the country, although one population was found inland (Fig. 1C).

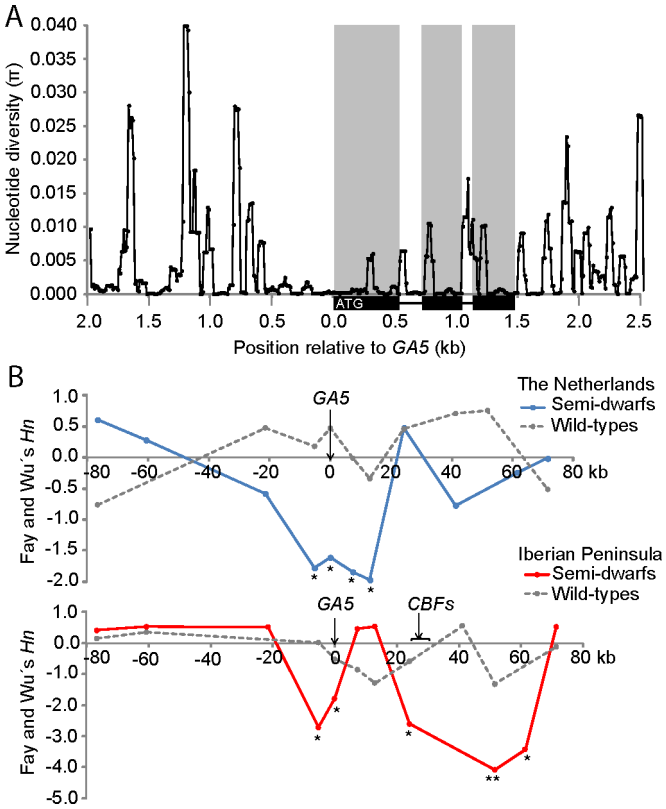

Fig. 4. . The *GA5* locus shows signatures of natural selection. (A) Nucleotide-sliding window analysis of nucleotide diversity (π) across the *GA5* locus in 505 *Arabidopsis* wild accessions. (B) Fay and Wu's *Hn* analysis across the *GA5* genomic region in populations containing semi-dwarfs from The Netherlands (blue), Iberian Peninsula (red), and populations of normal size (grey). Asterisks denote statistical significance *P<0.05, ** P<0.01.

Descriptions of the habitat of populations containing *ga5* semi-dwarf individuals show that they occur in multiple diverse environments where the species occurs. For instance, Dutch dwarf accessions were found in the anthropoid environments where Arabidopsis grows including urban (street populations) and rural (road and field sides, SI Appendix Fig. S2)habitats. However, in the Iberian Peninsula and Central Asia, semi-dwarfs occurred in more natural environments, including Mediterranean forests and mountain wet grasslands (SI Appendix Fig. S2). This wide geographic and ecological distribution indicates that *ga5* semi-dwarfism does not show a strong geographic structure and is not associated with a single and common climatic factor across its distribution range.

Identification of multiple *GA5* loss-of-function alleles.

To determine the putative mutations causing semi-dwarf phenotypes, we sequenced the *GA5* gene (~1.5 kb) in 59 semi-dwarf accessions collected world-wide and 135 non-dwarf individuals, which were collected from the same population or geographic region as the semi-dwarfs identified. For the Dutch OW and Sch populations, the ~1 kb *GA5* region, spanning semi-dwarf causal mutations, was sequenced in 16 semi-dwarfs and 77 wild-type individuals. Collectively, sequencing data identified 21 different mutations which were predicted to cause *GA5* loss-of-function alleles in semi-dwarf accessions (Fig. 2). These mutations were classified in six loss-of-function classes according to their nucleotidic nature. First, non-sense mutations causing premature stops codons were found in Kas-2 and Sparta. Second, missense mutations were found close to the conserved metal binding sites of *GA5* in an Iberian (Mar-1, Mar-3 and Mar-11) and a Scandinavian (Var 2-1 and Var 2-6) population, which might underlie their *ga5*

phenotype. Besides, the Mdc-10 and Mdc-53 semi-dwarf accessions also carried missense mutations in GA5 conserved domains. Third, a single substitution in the donor splice site of the first intron was found in all Dutch semi-dwarf accessions. This affects normal *GA5* splicing generating a truncated GA5 protein. Forth, seven small insertions (Cat-0, Dja-1 and Pak-3) or deletions (Cat-17, Cat-23, Cat-43 and Sus-1) were predicted to cause frameshifts and truncated GA5 proteins. Fifth, a transposon insertion, with high similarity to *At4g04410,* was identified in the MdcA-60 accession. Finally, several large deletions (> 20 bp) were found in some accessions. These included a 29 bp deletion in the first exon of Kl-2 (Germany) and a 444 bp deletion spanning part of the second exon and the complete third exon of accession T1080 (Sweden) (SI Appendix Fig. S3 and S4, Table S3). This deletion was first detected by the absence of sequence coverage in the 1001 genomes data (www.1001genomes.org) and further confirmed by extensive PCR amplifications (SI Appendix Fig. S3 and S4, Table S3). In addition, large *GA5* deletions of several kb were found in the Veg 1-1, Kyr-2, and YGU accessions. These deletions included not only the coding region but also the promoter (SI Appendix Fig. S3 and S4, Table S3) and were associated with absence of *GA5* expression in Kyr-2, Veg 1-2 and YGU.

Sequencing analyses indicated that most populations containing semi-dwarf individuals carry a single loss-of-function mutation in all dwarf plants (e.g. OW-0 in Fig. 2). However, two Iberian populations (Cat and Mdc) segregated for four independent *GA5* loss-of-function mutations (Fig. 2). One allele appearing more frequently as it was present in eight Cat individuals out of 22 sequenced samples. On the other hand, most *GA5* loss-of-function alleles appeared distributed in a single wild population, with the exception of the splicing site mutation widely distributed across The Netherlands. Analysis of the sequence data from the '1001 genomes project' detected four other putative semi-dwarf accessions from South Sweden (Sim-1, TV-22, TV-30 and TV-7), as they carry the Var 2-1 missense mutation. This result suggests that Var-2 missense loss-of function allele might be widely distributed at a local scale since Var, Sim and TV accessions originate from the same South-Swedish coastal area (SI Appendix Fig. S5 and Table S4).

Genome Wide Association Study (GWAS) for plant height.

Since several of the *ga5* semi-dwarf accessions identified in this study (Tha-1, Sparta, Var 2-1 and T1080) were included in the Arabidopsis Hapmap experimental population (15), we tested if the *GA5* locus could be detected by GWAS mapping. Measurements of plant height in 345 accessions of this collection showed a large amount of natural variation and high broad sense heritability (h_b^2=0.80) (Fig. 3A). However, no marker was significantly associated (P>0.05 with Bonferroni correction for 214,000 markers; SI Appendix Fig. S6) with plant height, the largest association was detected on chromosome 4, ~0.3 Mb away from *GA5* (P=3x10^{-5}; Fig. 3B). Analysis of Linkage Disequilibrium (LD) showed a complete LD decay 10 kb upstream and downstream of *GA5* (Fig. 3C), thus excluding the linkage of the observed association with *GA5*. By contrast, a significant association was detected when all four *GA5* loss-of-function alleles were combined as a single non-functional haplotype (P= 2.7×10^{-14}). Therefore, despite the strong effect of natural *GA5* loss-of-function alleles on plant height, GWAS was unable to detect this locus, due to the low frequency of semi-dwarf accessions and their multiple independent causal mutations.

***GA5* phylogeny and population structure.**

We determined the genetic relationships among the semi-dwarf accessions using a structure analysis with 117 genome-wide SNP markers already available (19, 20) or developed in this work. Structure analysis of these accessions found five distinct genetic groups that closely corresponded to the geographic regions of origin of the semi-dwarf accessions (Fig. 3D and SI Appendix Fig. S7) in agreement with the strong global geographic structure described in Arabidopsis (20). In all cases, semi-dwarf accessions were genetically more related to the non-dwarf individuals from the same population and region than to any other accession, indicating the independent origin and expansion of semi-dwarfs in these regions. In most populations containing *ga5* semi-dwarfs where five or more individuals were collected, wild-type *GA5* alleles were found within the population except for the Central Asian populations Dja and Sus, in which all individuals were semi-dwarf. Interestingly, Dja-1 and Sus-1 accessions carried different *GA5* loss-of-function alleles (Fig. 3D) regardless of the overall low genetic variation present in Central Asia (21). It is also remarkable that different *GA5* loss-of-function alleles were found in the Iberian Cat and Mdc populations together with wild-type alleles (Fig. 3D). In contrast, semi-dwarf genotypes in Dutch populations were very similar and carried the same loss-of-function mutation (SI Appendix Fig. S8).

Network analysis of the 33 different *GA5* haplotypes detected within the genomic *GA5* sequence identified a common *GA5* functional haplotype which showed a world-wide distribution (Fig. 3E and SI Appendix Table S5). Twenty other *GA5* haplotypes were connected to this frequent haplotype by fewer than five mutational steps and were distributed in all geographic regions. The central network position of the most frequent haplotype suggests that this is the oldest *GA5* allele, from which most other haplotypes may have derived by a small number of mutations (Fig. 3E). Furthermore, 14 additional low frequency haplotypes, which include only Iberian and Asian haplotypes (Cat, Mdc, Mar, Kas, Pak and Fuk), were separated from the main node of the network by two long related branches. Loss-of-function *GA5* haplotypes appeared evenly distributed within this network, and all but one of these alleles were connected by a single mutational step to their presumably ancestral haplotype. In addition, all loss-of-function haplotypes occupied branch-end positions in this network. Therefore, independent *GA5* loss-of-function alleles seem to be generated in multiple genetic backgrounds but they have not produced derived haplotypes (Fig. 3E).

Signatures of selection at the *GA5* locus.

To estimate the amount and pattern of nucleotide diversity in the *GA5* gene we analyzed the SNP data from 512 accessions available from the '1001 genomes project' *GA5* shows lower nucleotide diversity within coding regions than introns (Fig. 4A). Total nucleotide diversity (π = 0.0017, SI Appendix Table S6) was lower than the average nucleotide diversity reported in previous studies (0.0081 for centromeric and 0.0059 for non centromeric regions (22)). *GA5* also presents a low ratio of non-silent to silent polymorphism (π(ns)/π(s)=0.132), which is consistent with a signature of purifying selection, as previously suggested for rice GA biosynthesis genes (23). In addition, significant negative values for Tajima's D at non-synonymous sites (D_n) were detected in both the aforementioned 512 accessions (D_n=-2.289 p<0.01), as well as in the more than 100 accessions used in the present study (D_n=-1.987 p<0.05) including semi-dwarf haplotypes (SI Appendix Table S6). Overall, this pattern is compatible with the occurrence of purifying selection, in which polymorphisms leading to amino acid substitutions are maintained at low frequencies.

To test if positive selection may have contributed to an increase of *GA5* loss-of-function alleles, we searched for molecular fingerprints of recent selective sweeps over a region of 80 kb upstream and downstream of *GA5* in two populations from two different regions. These Cat (Iberian Peninsula) and Ow/Sch (The Netherlands) populations were selected because they contain a moderate frequency of *GA5* loss-of-function alleles. One additional population that does not contain semi-dwarf individuals from each of the regions was analyzed as control. Significant negative values of the normalized Fay and Wu's H statistics were found around *GA5* in the Cat and Ow/Sch populations containing

semi-dwarfs ($0.019<P<0.05$) (Fig. 4B), which is consistent with an excess of derived high-frequency mutations that commonly accompanies selective sweeps. We also detected negative values for the Fay and Wu's H_n statistics in the semi-dwarf Iberian Peninsula population around the *CBF* cluster involved in cold acclimation, for which natural variation has been reported (Fig. 4B) (24). This pattern was absent in populations without semi-dwarfs from the same regions (Fig. 4B). These results suggest that positive selection might contribute to increase the frequency of *GA5* loss-of-function mutations under particular environments, although drift and relaxed purifying selection could also contribute to a high frequency of *GA5* loss-of-function alleles in some other populations.

DISCUSSION

In this study we have shown that Arabidopsis semi-dwarf genotypes are relatively frequent in natural populations of different regions in the world and mostly caused by mutations in the *GA5*/*GA20ox1* gene . These results evidence a rather simple genetic basis for plant height, but its multi-allelic bases hampered *GA5* detection by GWAS mapping. Interestingly, *GA5* behaves as a functional orthologue of the green revolution genes of rice *SD1* and barley *Sdw1*/*Denso*. This result points to a conserved evolution for this common trait in crop and wild plant species. Thus, GA 20-oxidase is identified as a hotspot for phenotypic variation in plants (25), and illustrates the usefulness of the analysis of Arabidopsis natural variation to find genes of interest for plant breeding. The observation of major phenotypic changes caused by a large number of independent mutations resembles the situation found for the *FRIGIDA* gene of Arabidopsis involved in flowering time, another adaptive trait, which indicates that this pattern is not unique but rather common (17). As previously reported for *FRI* and *FLC* flowering genes, most *GA5* haplotypes show a sub-regional or local distribution, but the number of independent functional alleles was significantly larger in the Iberian Peninsula than in northern and central Europe, in agreement with the overall larger Iberian diversity (17, 21, 26).

Our study supports that different evolutionary forces might contribute to the occurrence of *GA5* loss-of-function alleles in nature.. The relatively high frequency of several *GA5* loss-function alleles in Central Asia and within local populations in The Netherlands, Central Asia and Iberian Peninsula suggests an advantage or neutrality. This is especially the case in some populations where multiple mutations have occurred and are still present. The wide geographic distribution of the same *GA5* allele found in many locations of The Netherlands separated more than 100 km indicates that this allele is spreading, further indicating the absence of deleterious effects. In addition, phenotypic characterization of *GA5* semi-dwarf accessions did not detect any strong negative effect on adaptive and fitness traits, which suggests that these alleles do not display any general obvious negative pleiotropic effect or trade-off. This result is in agreement with the phenotypes described for artificially induced *GA5* loss-of-function mutants, which show similar seed yield than wild-type accessions (8). This lack of effect on seed production is probably due to expression of GA20ox paralogues, mainly *GA20ox2* (8). Similarly, *GA20ox2* mutations in rice and barley do not display trade-offs (5, 6, 27). By contrast, mutations in early steps of GA biosynthesis have been associated with negative pleiotropic effects, such as the absence of seed germination shown by *ga1* null mutants or the reduced fertility and altered flower development observed even in leaky *GA1* alleles (13). A similar situation has been reported in rice where the effects derived from mutations on genes involved in early steps of GA biosynthesis were less favorable for crop production compared with mutations on rice *GA20ox2* (28).

Our analyses suggest that both negative and positive selection may act on *GA5* loss-of-function alleles. The conditional negative effect of these alleles is suggested by the low frequency of most loss-of-function alleles, and by the fact that they are not maintained long enough to derive new haplotypes. Hence, such alleles seem to be transiently maintained in nature. In addition, such potential negative effect of *GA5* loss-of-function alleles is also suggested by purifying selection inferred from the low ratio of replacement to silent polymorphisms and negative Tajima's D_n values, in agreement with previous reports in rice (23). In contrast, positive selection might contribute to transient increases in the frequency of loss-of-function alleles in certain populations, as suggested by the negative values of Fay and Wu's H_n tests across the *GA5* locus for the two tested populations segregating for semi-dwarf individuals. Remarkably, this pattern is absent in populations of normal size plants from the same regions. Therefore, we reason that allelic variation at *GA5* locus might be maintained in nature by antagonist pleiotropy, (*i.e.* reversed fitness effects in different environments) (29). However, we cannot discard that *GA5* variation shows conditional neutrality in other populations (*i.e.* loss-of-function alleles might be neutral in some environments but deleterious in others). Neutrality tests should be considered carefully due to the complex demographic history of Arabidopsis populations in the wild. Furthermore, the population genetic analysis is agnostic to the local extinction or re-colonization dynamics of Arabidopsis populations. The identification of signatures for selection using genome-wide screens may be hampered by the occurrence of different loss-of-function *GA5* alleles under positive selection, a situation that also affected GWAS mapping.

It remains to be determined which are the environmental cues that could contribute to an increase in the frequency of *GA5* loss-of-function alleles since these mutations appear distributed in a wide range of anthropoid and natural environments. It has been previously shown that the short plant height phenotype caused by the well-known *erecta* loss-of-function mutation provides fitness advantage in static landscapes. On the contrary, the *erecta* frequency was reduced under disturbed environments (30). Analogously, it can be speculated that environmental stability might favor *GA5* semi-dwarf individuals. Conclusive demonstration about positive, negative or neutral fitness effects of *GA5* loss-of-function alleles depending on the environment will require further analyses under different natural conditions to elucidate the evolutionary forces driving *GA5* variation and its ecological significance.

MATERIALS AND METHODS

Plant Material and growth conditions. Stock numbers and detailed information of accessions used in this work are listed in SI Appendix Table S1. For allelism tests, semi-dwarf accessions were crossed with L*er* and *ga5* (13). To facilitate the allelism tests, male sterility based on the *ms1* mutant (31) was introgressed into the *ga5* background. Plants were grown under greenhouse conditions at 16 h light, 22°C/18ºC day/night cycles. For all experiments, seeds were stratified in water at 4°C for 4-6 days prior to germination. Ten repetitions per genotype (cross) were conducted. All crossed accessions are listed in SI Appendix Table S2. The Ooij, Schar, Hoor, Haarl, and Oosth Dutch semi-dwarf populations and the Mdc Iberian semi-dwarf population were found in the course of our studies. Allelism was concluded based on sequence data that correlated with the semi-dwarf phenotypes and haplotypes tested before in allelism tests. Phenotyping for plant height and height up to first silique was conducted two weeks after flowering because both traits did not change after that date (SI Appendix Fig. S9). In cases of extreme flowering lateness, plants were vernalized for six weeks. Flowering time was recorded as days after germination until the first opened flower. Branch number was scored as the number of axillary stems grown from the rosette.

Sequencing of *GA5* gene and genotyping. Genomic DNA was isolated from leaf material using the BioSprint workstation (Qiagen). Primers used for *GA5* sequencing are detailed in SI Appendix Table S7. PCR reactions were performed using LA Taq DNA polymerase (Takara) following manufacturer's instructions. Sanger sequencing of purified PCR products was made by the Max Planck Genome Center Cologne. GenBank accession numbers of DNA sequences generated in this work are listed in SI Appendix Table S5. SNP genotyping of new accessions collected in this study was done as described in

previous works (19, 20) using the genotyping facility service of the University of Chicago.

Statistical analysis. Descriptive statistics, t-tests, tukey test, and principal component analysis were conducted with R. The method of EMMAX was used for GWAS (32) using kinship matrix to correct for population structure. Linkage disequilibrium analysis was performed with the R package LD heatmap (33).

Structure analysis. Population structure was inferred using model-based clustering algorithms implemented in the software STRUCTURE , using the haploid setting and running 20 replicates with 50,000 and 20,000 MCMC iterations of burn-in and after-burning length, respectively (34). To determine the K number of significantly different genetic clusters, we applied the $\triangle K$ method in combination with the absolute value of ln P(X |K) (35).

Population genetics. Fay and Wu's H statistics and haplotype network. Population genetics analyses were conducted with the software DnaSP (5.10) (36). The normalized Fay and Wu's H was performed as described (16) in populations containing semi-dwarfs from The Netherlands and Iberian Peninsula (SI Appendix Table S8). Representative accessions of different populations from Central Spain, with no semi-dwarfs, were used as control (SI Appendix Table S8). For the Dutch control population, accessions from a rural area northeast of Wageningen were collected with no prior knowledge of semi-dwarfness occurring in this population (SI Appendix Table S8). The sequences of *GA5* (*At4g25420*) and flanking genes were obtained after specific PCR amplification from genomic DNA and sequencing in ABI 3730XL automated sequencers (Applied Biosystems) (SI Appendix Table S7). Sequences were aligned with ClustalW (37) and manually inspected. *Arabidopsis lyrata* sequences were obtained by BLAST search (http://www.phytozome.net/) and used as out-group to assign ancestral and derived states to SNP variants. To assess the statistical significance of Fay and Wu's H, we computed 10,000 coalescent simulations in DnaSP v.5.10 (36). The haplotype network of *GA5* was constructed using TCS1.21 (38) that implements a maximum parsimony method and excluding gaps as events in the analysis. Insertions and deletions in the semi-dwarf accessions were considered as single events and added manually to the haplotype network.

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